STUDY OF EXPRESSION OF CD 117 AND CD 34 IN PHYLLODES TUMOR OF BREAST AND ITS CORRELATION WITH HISTOPATHOLOGICAL GRADE

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M.D.Degree

in PATHOLOGY-BRANCH III

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DECLARATION

I solemnly declare that this dissertation entitled **"STUDY OF EXPRESSION OF CD 117 AND CD 34 IN PHYLLODES TUMOR OF BREAST AND ITS CORRELATION WITH HISTOPATHOLOGICAL GRADE"** was done by me in the Department of Pathology, Coimbatore Medical College, Coimbatore during the period of June 2014 to July 2016 under the guidance and supervision of **DR.C.LALITHA, MD.,** Professor and Head, Department of Pathology, Coimbatore Medical College, Coimbatore.

This dissertation is submitted to **The Tamilnadu Dr.M.G.R. Medical University,** Chennai towards the partial fulfillment of the requirement for the award of M.D., Degree in Pathology.

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Date: **Dr. R. ARTHI**

CERTIFICATE

This is to certify that the dissertation entitled **"STUDY OF EXPRESSION OF CD 117 AND CD 34 IN PHYLLODES TUMOR OF BREAST AND ITS CORRELATION WITH HISTOPATHOLOGICAL GRADE"** is a record of bonafide work done by **Dr.R.Arthi,** Post Graduate student in the Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore under the guidance and supervision of Dr.C.Lalitha, M.D., Professor and Head, Department of Pathology, Coimbatore Medical College and Hospital, Coimbatore in partial fulfillment of the regulations of Tamilnadu Dr.M.G.R. Medical University, Chennai towards the award of M.D.Degree (Branch III) in Pathology.

Dr. Edwin Joe, M.D.,B.L.,
Dean, Coimbatore Medical College,
Coimbatore.

Dr. C. Lalitha, M.D.,
Professor and Head,
Department of Pathology,
Coimbatore Medical College,
Coimbatore.

Coimbatore Medical College

COIMBATORE, TAMILNADU, INDIA - 641 014

(Affiliated to The Tamilnadu Dr. MGR Medical University, Chennai)

ETHICS COMMITTEE

CERTIFICATE

Name of the Candidate : DR. ARTHI. R

Course : M.D. PATHOLOGY

Period of Study : 2014 - 2017

College : COIMBATORE MEDICAL COLLEGE

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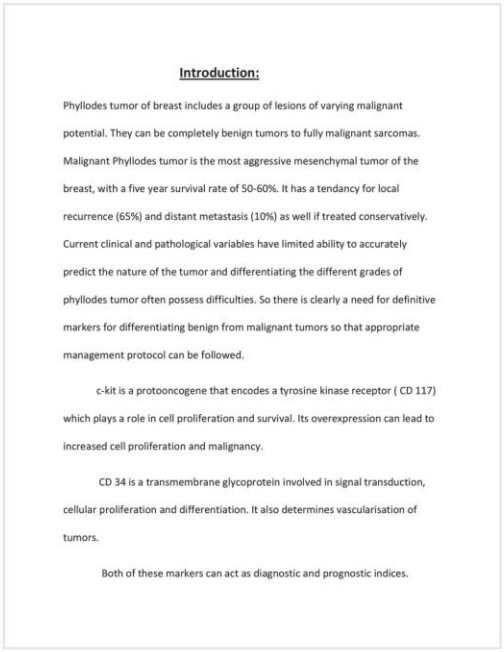


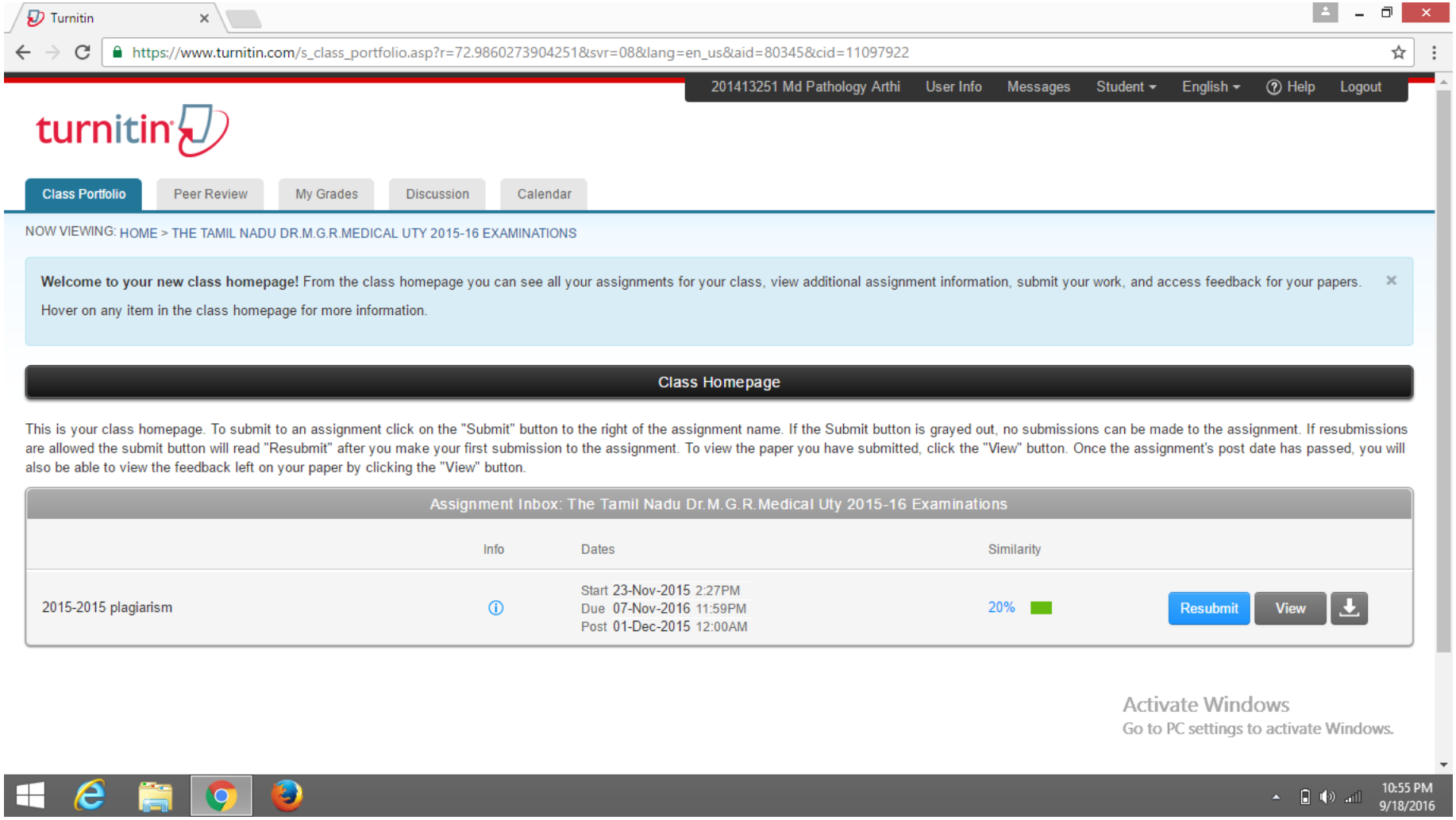
Introduction:

Phyllodes tumor of breast includes a group of lesions of varying malignant potential. They can be completely benign tumors to fully malignant sarcomas. Malignant Phyllodes tumor is the most aggressive mesenchymal tumor of the breast, with a five year survival rate of 50-60%. It has a tendancy for local recurrence (65%) and distant metastasis (10%) as well if treated conservatively. Current clinical and pathological variables have limited ability to accurately predict the nature of the tumor and differentiating the different grades of phyllodes tumor often possess difficulties. So there is clearly a need for definitive markers for differentiating benign from malignant tumors so that appropriate management protocol can be followed.

c-kit is a protooncogene that encodes a tyrosine kinase receptor (CD 117) which plays a role in cell proliferation and survival. Its overexpression can lead to increased cell proliferation and malignancy.

CD 34 is a transmembrane glycoprotein involved in signal transduction, cellular proliferation and differentiation. It also determines vascularisation of tumors.

Both of these markers can act as diagnostic and prognostic indices.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INTRODUCTION

INTRODUCTION

Phyllodes tumor of breast includes a group of lesions of varying malignant potential. They can be completely benign tumors to fully malignant sarcomas. Malignant Phyllodes tumor is the most aggressive mesenchymal tumor of the breast, with a five year survival rate of 50-60%. It has a tendency for local recurrence (65%) and distant metastasis (10%) as well if treated conservatively. Current clinical and pathological variables have limited ability to accurately predict the nature of the tumor and differentiating the different grades of phyllodes tumor often possess difficulties. So there is clearly a need for definitive markers for differentiating benign from malignant tumors so that appropriate management protocol can be followed.

The c-kit is a protooncogene that encodes a tyrosine kinase receptor (CD 117), which plays a role in cell proliferation and survival. Its overexpression can lead to increased cell proliferation and malignancy.

CD 34 is a transmembrane glycoprotein involved in signal transduction, cellular proliferation and differentiation. It also determines vascularization of tumors.

Both of these markers can act as diagnostic and prognostic indices.

With the need to differentiate benign from malignant tumors and also with the advent of specific therapy targeted at CD117, this study was conducted to explore the expression of CD117 and CD34 in phyllodes tumor of breast.

AIM & OBJECTIVES

AIM OF THE STUDY

To study the immunoexpression of CD 34 and CD 117 in phyllodes tumor of breast and correlate it with histopathological grade.

OBJECTIVES

1. To assess the expression of CD34 and CD 117 in phyllodes tumor of breast
2. To correlate the expression of these markers with the histopathological grading
3. To assess the usefulness of these markers in differentiating benign from malignant phyllodes tumor of breast so that appropriate treatment can be administered.

REVIEW OF LITERATURE

REVIEW OF LITERATURE

ANATOMY AND PHYSIOLOGY OF THE BREAST

The structure of human breast is a reflection of its special function, which is production of milk. The epithelial component of breast tissue is constituted by lobules, which is the site for milk production and it connects to ducts that open onto the nipple. These lobules and ducts are found to lie in a background of fibrous and adipose tissue. Adipose tissue makes up the main breast mass. The structure of the male breast is similar to that of the female breast except that it lacks the specialized lobules since there is no need for milk production.

Anatomically the adult breast lies on top of the pectoralis muscle over the ribcage. The breast tissue extends horizontally from the edge of the sternum to the midaxillary line. It is to be noted that a portion of breast tissue called the axillary tail of Spence extends into the axilla.

The breast tissue is surrounded by a thin layer of connective tissue known as fascia. The deep layer of the fascia lies immediately over the pectoralis muscle and the superficial layer lies immediately under the skin.

The skin covering the breast is just the same as the skin anywhere else on the body and has got sweat glands, hair follicles etc.

The blood supply for the breast is primarily from internal mammary artery which courses beneath the breast tissue proper. The lymphatic vessels of the breast course in a direction opposite to that of the blood vessels and drain into the lymph nodes. Most lymphatic vessels drain into the axillary lymph nodes while a few of the lymphatics drain into internal mammary lymph nodes, which are present deep to the breast tissue. Knowledge of this lymphatic drainage is important because when a breast cancer metastasizes it usually involves the first lymph node in the chain of lymph nodes. This is called the "sentinel lymph node" and a surgeon may remove this lymph node to check for metastases in a patient with breast tumor.

Physiologically the breast is an organ specialized for milk formation (lactation). Many additional changes are seen in the breast tissue during pregnancy and lactation due to the changes in hormones during those times.

PHYLLODES TUMOR

Phyllodes tumor of breast was first fully described in 1838 by Johannes Müller[1]. It was given the name cystosarcoma phyllodes to highlight the leaf-like pattern and fleshy gross appearance of the tumor. The other name commonly used for the tumor is periductal stromal tumor because of its origin from periductal stroma. PT is sub classified into three groups benign, borderline and malignant based on the histologic characteristics of the tumor.

CLINICAL PRESENTATION

Patients usually present with a firm to hard mass. No clinical feature can accurately distinguish between fibroadenoma, benign Phyllodes tumor, and malignant Phyllodes tumor[2]. Features favouring diagnosis of PT are tumor size more than 4cm and history of rapid growth of tumor. When there is sudden enlargement of a tumor that was stable for many years the possibility of tumor originating from a preexisting fibroadenoma or malignant transformation of a benign phyllodes tumor can be considered. Clonal analysis of a few tumors that were originally diagnosed as fibroadenomas but later recurred as phyllodes tumors suggest that phyllodes tumor can arise from a preexisting fibroadenoma [3].

It was found that the same allele of the X chromosome linked AR gene was inactivated in the fibroadenoma and PT samples from each patient.

Phyllodes tumors usually present as solitary unilateral masses. It rarely involves both breasts or can occur as multicentric tumor [4,5,6,7,8]. Coexistent fibroadenomatoid lobular hyperplasias are often noted in the surrounding breast tissue.

Phyllodes tumors can affect any age group from 10 to 86 years [9,10,11,12]. Most commonly affects women in the fourth decade, the median age being 45 years.

It is only rarely seen in women younger than 30 years. A few cases have been reported in adolescent females and most of the cases were benign, only occasional cases of malignant phyllodes have been reported in the patients of this age group [16, 17,18,19,20,21,22].

The tumors can range in size from 1-20 cms but they are mostly around 4 cms in size. Malignant tumors are generally larger in size but malignancy has been reported in lesions as small as 2 cms in size and some of the large tumors have proved to be benign. Large tumors can extend to the overlying skin or chest wall [25].

Mammography mostly reveals a lobulated and well-defined opaque mass. A few cases have indistinct border. In ultrasound the tumor appears well circumscribed but not homogenous due to the presence of cystic spaces and epithelium-lined clefts [26, 27].

Calcifications can be rarely seen in both benign and malignant lesions [27,28]. Ultrasonography and mammography are not very useful in distinguishing benign from malignant phyllodes tumor. MRI is more reliable for distinguishing benign from malignant tumor [29,30].

The role of flow cytometry analysis of ploidy and S-phase in the classification of PTs is uncertain. S phase and ploidy analysis by flowcytometry is only of limited help in the classification of phyllodes tumor.

Complex karyotypic abnormalities have been observed in malignant phyllodes tumor via Cytogenetic studies [35]. Recurrent tumors have also been found to have the same genomic abnormality as the primary [39].

Biochemical analysis has revealed expression of PR in the stroma of phyllodes tumors but only a few of the tumors show expression of ER [40]. Insulin-like growth factor II (IGF-II) produced by a malignant PT was

associated with hypoglycemia in one case [41]. IGF-II has also been occasionally detected immunohistochemically in tumor stromal cells.

GROSS PATHOLOGY

The tumors are generally encapsulated circumscribed single or multinodular masses. Even Phyllodes tumors with microscopically invasive borders may appear circumscribed grossly.

The cut surface of the tumor is firm, bulging, gray to tan tissue. Areas of hemorrhage and necrosis can be noted which are most commonly seen in malignant tumors. Rarely large benign tumors may also show these changes. Cysts containing keratotic material are rarely seen. Very rarely phyllodes tumor can have a cystic component mimicking a cystic papilloma [(42)]

MICROSCOPIC PATHOLOGY

The tumor originates from periductal stroma. Histologically most Phyllodes tumors have a heterogeneous appearance and only a few tumors have the structure of an intracanalicular fibroadenoma with exaggerated stromal cellularity. In many cases there is ductal epithelial hyperplasia, which masks the intracanalicular pattern.

Many features help in distinguishing a fibroadenoma from a benign Phyllodes tumor. Unlike fibroadenomas, Phyllodes tumors have increased cellularity of the stromal component. In some cases stromal cellularity is more intense in areas adjacent to epithelial components (periductal stroma). Mitotic activity is also increased in the same pattern; on the other hand fibroadenomas do not reveal any stromal mitoses. PTs may have a nodular structure with prominent periductal stromal proliferation. These are called **periductal stromal tumors** and further classified as periductal stromal hyperplasia or periductal stromal sarcoma [(43).] These tumors are highly prone for recurrence with features of phyllodes tumor being noted in the recurrent tumor. Yet a significant number of PTs show no zonal stromal distribution.

The presence of epithelial-lined clefts is a feature seen in PTs and rarely these clefts are dilated and condensation of the stroma immediately surrounding it can be seen. These clefts can also be seen in fibroadenomas. The intracanalicular pattern of fibroadenomas may mimic the clefted architecture of PTs and sometimes differentiating the 2 tumors can be difficult. This difficulty is particularly common giant fibroadenomas where tumor size and clefts that are made out grossly suggest PT. Microscopically the stroma in intracanalicular fibroadenomas is hypocellular and uniform.

Myxoid change in stroma can be seen in both fibroadenomas and PTs. It is mostly homogeneous in fibroadenomas and is patchy with degenerative changes in PTs. Pseudoangiomatous stromal hyperplasia (PASH) can occur in PT and in some cases PASH can be a predominant feature. Occasionally multinucleated giant cells are found in stroma of a PT with PASH features. These giant cells can show lymphophagocytosis. They may express markers such as CD68 which is histiocytic marker as well as p53 and Ki67.

Stromal cellularity in phyllodes tumor can be heterogeneous with areas indistinguishable from fibroadenoma that juxtapose sharply on areas with increased cellularity. These features can make one to conclude that the PT arises from a fibroadenoma when actually these features are a part of some PTs. In certain tumors sampled by fine needle aspiration or needle core biopsy, variations in pattern and stromal cellularity may create difficulties in the classification of the tumor. Inappropriate classification and inadequate sampling may be the causes for malignant behavior and metastases from a benign tumor. Excisional biopsy is the best choice for grading a PT. Grading is based **on stromal cellularity, mitotic activity, and microscopic character of the tumor periphery.**

The PT is sub classified into three groups. Distinguishing benign PT from low-grade malignant PT is important because the benign tumors do not metastasize, they have a low risk for local recurrence, the time interval to recurrence is longer, and initial recurrences are histologically benign most of the time. Low-grade malignant PTs have very early local recurrence and the recurrences are mostly histologically high grade.

A benign PT has very few mitoses - usually one to two per 10 hpf, moderate to marked cellular overgrowth and moderate cytologic pleomorphism[141]. Stromal expansion and cellularity are maintained uniform throughout the lesion but these characteristics can be heterogeneous[141]. There is a correlation between the degree of epithelial proliferation and the appearance of the stroma. Epithelial hyperplasia which is not very conspicuous in an ordinary benign PT can occasionally be well identified in some cases. Although the border is usually well defined invasion can be present , at times in the form of nodules of benign tumor around the main tumor. Lipomatous and osseous metaplasia can be seen in the stroma of a benign PT. Multinucleated giant cells with hyperchromatic nuclei can occasionally noted in the stroma of PT. These cells have been found to be immune positive for p53 and Ki67. Rarely stromal myxoid changes can be noted in a benign

PT. Pseudoangiomatous hyperplasia of stroma can be seen in both benign and malignant PTs.

A malignant PT features a high degree of hypercellular stromal overgrowth[141]. In many cases this can lead to significant separation of epithelial elements, high proliferative activity in the stroma, more than five mitoses per 10 hpf and an invasive tumor border[141]. Stromal overgrowth and cellular atypia is common in these tumors. Stromal overgrowth has been defined as marked stromal proliferation to the point where the epithelial component is absent in at least 1 low-power field (×40)[140]. Rarely the stroma can contain heterologous sarcomatous elements like angiosarcoma, liposarcoma, chondrosarcoma etc. [45,46,47,48].

Borderline tumors show a circumscribed or invasive border, two to five mitoses per 10 hpf, and moderate amount of stromal cellularity that is mostly heterogeneously distributed admixed with hypocellular areas. The stroma containing spindle cells may mimic fibromatosis or low-grade fibrosarcoma or it may show pseudoangiomatous hyperplasia. Occasionally cartilaginous, osseous or lipomatous metaplasia have been found in borderline PTs. Epithelial hyperplasia is noted in many tumors. This is seen as varying increase in the thickness of the epithelium lining the slit-like space and the epithelium is usually cuboidal or columnar. Increased thickness due to several layers of cells and hyperplasia of

myoepithelial cells is often found. This may progress to papillary and cribriform hyperplasia. The severity of epithelial hyperplasia correlates with cellularity of the stroma and mitotic count but this is not always the rule. Atypical epithelial hyperplasia can be extensive in some cases leading to mistaken diagnosis of intraductal carcinoma. Sometimes the stromal component can be misdiagnosed as reactive and the diagnosis of phyllodes tumor can be overlooked. Tumors in which phyllodes pattern of growth is obscured by an uncommon epithelial distribution usually are misinterpreted as papillary neoplasms or as adenosis tumors.

Very rarely the epithelial abnormality can reach a level that can be considered as intraductal carcinoma and finding intraductal or invasive duct carcinoma in phyllodes tumor is very uncommon [49,50]. In situ and invasive duct and lobular carcinoma can also be seen PTs [51].

Squamous metaplasia, which can occur in benign and malignant PTs, can be seen in about 10% of cases. Aspiration from a cystic area of squamous metaplasia can lead to a mistaken diagnosis of squamous cyst [52]. Apocrine metaplasia has also been reported occasionally in PTs [53,54]. Lobules showing proliferative changes like sclerosing adenosis can sometimes be seen in PTs. The presence of lobules especially with hyperplasia can lead to mistaken diagnosis of fibroadenoma when the stroma is not very cellular. In some instances the proliferation of

epithelium in the form of adenosis and papillary hyperplasia can be so extreme that it masks the underlying PT which may go unrecognized and recur.

Very rarely stromal cells in benign phyllodes tumor can show intracytoplasmic inclusion bodies like in infantile digital fibromatosis [55]. Electron microscopy in such cases showed a combination of fibroblasts and myofibroblasts. The intracytoplasmic inclusions were associated with cytoplasmic microfilaments that formed tadpole-like structures. These cells stained very weakly for actin by immunohistochemical method and the inclusion bodies did not show any reactivity. Post pretreatment with potassium hydroxide in 70% ethanol and also 0.1% trypsin both the cells and inclusions stained strongly for actin.

Ductal elements can be seen in locally recurrent PTs in the breast or chest wall. With some rare exceptions most of the metastatic PTs at distant sites have entirely the stromal component only. Few case reports have shown epithelial component in lung metastases [56,57]. One of these represents inclusion of pulmonary tissue in the metastatic deposit [58].

Exceptional primary malignant PTs exhibiting liposarcomatous differentiation with adenosis-like glandular component are on record. These features were also seen in lung metastases. The adenosis-like

components in the primary tumor and in the metastases were immunopositive for gross cystic disease fluid protein 15 (GCDFP-15). The cells in these glandular structures were surrounded by myoepithelial cells, which were immunopositive for actin.

Because most of the malignant PTs are high-grade spindle cell lesions with fibrosarcoma like pattern, it is the most common pattern seen in metastatic lesions. Rarely locally recurrent tumor or the metastatic tumor may exhibit heterologous differentiation that was not noted in the primary tumor [58]. Rare heterologous sarcomatous components in the primary tumor like lipo- , osteo- [60], chondro- [59], and leiomyosarcoma can be seen in metastases. Rhabdomyosarcoma has been observed in the lung metastases from a malignant PT that had rhabdomyosarcomatous element [(45)]. Metastases from a PT with liposarcomatous element had mainly immature lipoblasts and very few adipocytes [62].

Adequate sampling is important with at least 1 block for every 1 cm of maximal tumor dimension.

IMMUNOHISTOCHEMISTRY

The stroma is vimentin-positive. Actin, CD34, and desmin positivity are present in the stroma of cases that exhibit myoid or pseudoangiomatous stromal differentiation of myofibroblasts [63,64]. Stromal cells are occasionally positive for S-100. Expression of c-kit, a protooncogene that codes for tyrosine kinase receptor (CD117) has been observed in the stroma of Phyllodes tumors [62]. A significantly higher frequency of CD117 immunoreactivity has been observed in high-grade malignant tumors than in benign tumors . It has also been shown that vascular endothelial growth factor (VEGF) is also expressed in phyllodes tumors. Expression of p53 in more than 10% of stromal cells has been observed more in malignant PTs than in benign PTs [68]. Expression of CD10 in fibroepithelial tumors has also been studied. CD10 positivity has been found in fibroadenomas, benign PTs, borderline phyllodes tumors and malignant phyllodes tumors. But the reactivity is found in more number of malignant phyllodes tumors compared to others.

Immunomarker of stromal proliferative activity can be helpful in distinguishing fibroadenomas from phyllodes tumors and also in the classification of phyllodes tumors. The immunohistochemical expression of Ki67 detected using MIB1antibody helps in distinction between

malignant and benign phyllodes tumors [71]. The proportion of Ki67-positive cells was found to be higher in malignant phyllodes tumors compared to the benign ones. Ki67 immunopositivity in more than 10% of stromal cells has been reported in many malignant PT compared to benign tumors[68]. The Ki-67 index has also proved to be helpful in distinguishing fibroadenomas from benign phyllodes tumors in females 25 years of age or younger. A significant difference in the Ki67 reactivity in the stroma and epithelium of phyllodes tumors and fibroadenomas has been found with the Ki67 indices being lower in fibroadenomas. There was also a significant correlation between the stromal and epithelial Ki67 activity. Further evaluation is needed to find out about the heterogeneity of Ki67 expression in Phyllodes tumor and to determine whether a needle core biopsy specimen is reliable for assessing the expression of this immunomarker.

It has been found that PTs contain a much higher concentration of endothelin-1 compared to fibroadenomas [73]. This vasoactive peptide stimulates synthesis of DNA in vascular smooth muscle cells and breast stromal cells [74]. Immunohistochemical studies have revealed that endothelin-1 is found in the epithelium of PTs and that it is absent in the stromal cells of these tumors [73]. This feature suggests that endothelin-1 produced by the epithelial cells of PTs may have a paracrine action in

stimulating the proliferation of stromal cells. Routine histologic and morphometric studies have shown that mitotic activity tends to be higher in stroma close to the epithelium in Phyllodes tumors rather than at sites distant from epithelium and this is also suggests the paracrine function of PT epithelium [75].

Tenascin an extracellular matrix glycoprotein that inhibits interactions between cells and between cells and stroma has been found in some fibroepithelial tumors, restricted subepithelial zone of stroma in normal breasts and in cases of fibroadenomas but it is more diffusely seen in the stroma of PT [76].

ELECTRON MICROSCOPY

Ultrastructurally the stroma of PT is made of cells with features of fibroblasts and myofibroblasts that are similar to the usual cellular constituents of the breast stroma. Electron dense cytoplasmic bodies at times with a crescent shape have been described as a unique feature by some authors [77]. These structures are of lysosomal origin, and they are found in higher number in malignant tumors. Various other types of cytoplasmic inclusions have also been described [78,79]. Intermediate filaments and dense bodies have been observed in myofibroblastic cells.

Electron microscopy has not revealed much of unusual features in the epithelial component of PTs.

CYTOLOGY:

A cytologic diagnosis of PT can be made when the aspirate has an epithelial component like a fibroepithelial neoplasm and in addition has excess bipolar stromal cells. It's the stromal cells with cytoplasm rather than the naked bipolar nuclei that are typical of Phyllodes tumors [80]. Cellular stromal fragments are useful in differentiating Phyllodes tumor from fibroadenoma [81,82]. Aspiration cytology is not a very reliable procedure for diagnosing PT in certain situations [83].

Tumors with prominent epithelial hyperplasia may yield an aspirate or biopsy sample with obscured stromal element and this can lead to mistaken diagnosis of a carcinoma [84] or a fibroadenoma, in case the sample is obtained from a tumor, which is heterogenous and has bland epithelium with sparse stromal cells. Scarce single epithelial cells, cohesion of epithelial cells and polarity are epithelial features seen in PT rather than carcinoma in those specimens [84]. The aspirate from malignant PT mostly contains cellular stromal fragments made of atypical cells along with mitotic figures. Fragments of stroma containing adipose

differentiation may be seen in the cytologic specimen from a PT having adipose or liposarcomatous differentiation [85.]

TREATMENT AND PROGNOSIS

Classifying PT into benign, borderline and malignant gives an estimate of the clinical course based on the histologic features of the tumor. Benign phyllodes tumors do not metastasize and have a low probability for recurrence after excision[86]. Low-grade malignant and borderline PTs have a minimal probability of metastasis; such tumors however are more likely to recur locally than a benign Phyllodes tumor. Metastasis occurs in about 25% of high-grade malignant tumors and these tumors also have a very high probability of local recurrence. Recurrences tend to occur earlier with high-grade malignant tumors than benign or borderline tumors post initial treatment. Axillary lymph node metastasis is noted in less than 1% of high grade tumors [87.] One case of Rotter lymph node showing metastatic PT has been reported [88.] Classification of PT as benign, borderline and malignant correlates with local recurrence in women who did not undergo surgery.

The basis of therapy is complete excision to prevent any local recurrence [91,92,93,94]. Features that favour local recurrence are incomplete excision of the tumour, an invasive border and secondary tumor nodules

at periphery. Primary tumor size could be a factor involved in the success of local excision because a wider margin resection is possible when tumor size is small [91]. Local recurrence is dangerous especially because of the tendency of some tumors to become a higher-grade lesion during recurrence than the corresponding primary tumor and there is risk of chest wall invasion during recurrence.

Systemic metastases are not necessarily preceded by local recurrence in patients with malignant PT. However very rare instances of a benign or a low-grade malignant PT giving rise to metastases have almost always had local recurrences with higher grade malignant features before the appearance of systemic lesions. Almost half of the patients with malignant PT who develop metastases do not develop a local recurrence before systemic spread [95,96].

As the diagnosis of PT is not predicted clinically in a lot of cases, surgical excision may be incomplete initially and re-excision may be required. Both the primary excision and re-excision specimens have to be inked and margins should be thoroughly examined histologically. Mastectomy is indicated in cases of malignant PT. Axillary lymph node dissection is done if there is concurrent carcinoma in PT or in some other location in the same breast or when the lymph nodes appear to be clinically involved by the tumor.

Tumors with different types of stromal differentiation appear to have differences in prognosis as well. Many patients with osteogenic or chondrosarcomatous differentiation have developed systemic metastases [59,60] but most of the patients with liposarcomatous differentiation have had good prognosis [46,62,97,98].

Metastasis most commonly occurs in the lungs, bone, and heart [99]. One case has been reported with an uncommon instance of surgically resected metastatic PT in lung with intravascular dumbbell extension through the pulmonary vein into left atrium [100]. Virtually metastasis can occur in any organ but many of these metastatic sites are not evident antemortem. Exceptional sites with clinically detectable metastasis are the mandible [101], maxilla [102] and brain [103,104.]

The 5-year survival rate for phyllodes tumor is around 90% [104]. Local recurrences, which are seen in about 30% of cases and metastases that occur in about 10% of cases, are usually found within 3 years of primary treatment [105]. Although occasionally instances of late recurrence have also been reported. Most of the deaths due to metastatic PT occur within first 5 years of diagnosis [106]. Almost all deaths occur in patients who have high-grade primary tumors or who develop high grade recurrences. These high-grade tumors are typically characterized by stromal overgrowth, invasive borders, frequent mitoses and cellular

pleomorphism [107,108]. It has been reported that all PTs that resulted in metastases have mitotic rates of at least 15 per 50 hpf either in the primary tumor or in the recurrences.

Metastatic PTs do not respond to most of the currently available chemotherapy and radiotherapy [108]. Prolonged remission has been reported in few patients treated with ifosfamide [107] and palliation has been reportedly achieved with combination chemotherapy and radiation in few cases [109].

The c-kit expression correlates with the grade of phyllodes tumor [66,67,110]. It has been reported that stromal c-kit immunoreactivity is an effective predictor of recurrence. The detection of c-kit expression in some Phyllodes tumors may be useful for the treatment of these phyllodes tumors with drugs that inhibit tyrosine kinase receptors [65].

PHYLLODES AND FIBROADENOMA

Phyllodes tumors were once taken to be the same as giant fibroadenomas as they grow rapidly, are larger in size than fibroadenomas and can have the same intracanalicular pattern as fibroadenomas. Often benign phyllodes tumor's slight increase in stromal cellularity can lead to mistaken diagnosis cellular fibroadenoma [111, 113].

It is morphologically very difficult to distinguish between the two in limited tissues like core needle biopsies [111, 113]. In one study fibroadenomas occurred simultaneously with phyllodes tumors in approximately 3% of cases. Hence the molecular profiles of phyllodes tumor and fibroadenoma are quite often compared and studied to get the explanation as to how their behaviours are so different in spite of being morphologically similar in many ways [115, 116].

Clonal analysis of the fibroadenoma as well as phyllodes tumor via polymerase chain reaction proved that fibroadenomas were polyclonal in both epithelium and stroma whereas phyllodes tumors were polyclonal only in epithelial cells and were monoclonal in stromal cells [115]. It has been suggested that the histogenesis of the two tumors is similar and in case of phyllodes tumor the neoplastic component is the stroma [115]. It has been further said that when monoclonal stromal cell proliferation is not likely, phyllodes tumor would start as a fibroadenoma with stromal cell mutations leading to phyllodes tumor [115]. Studies have lead to the idea that the phyllodes tumor is mainly a tumor of the stroma with the epithelial component not taking part in the tumorigenic process as the stroma keeps proliferating. In addition it is said that the fibroadenoma could be precursor lesion of the phyllodes tumor [116] a possibility, which till date, has not been entirely ruled out.

Many phyllodes tumors in addition show features of epithelial hyperplasia and ductal carcinomas [117], this has raised the question of how innocent is the epithelial component in reality. Comparative genomic hybridization (CGH) has shown gain of 1q and loss of 3p as the most frequent chromosomal abnormalities [117]. As this genetic profile suggested that the pathogenesis is similar in both phyllodes tumor and breast carcinoma, allelic imbalance (AI) assessments were done using microsatellites on chromosomes 1q and 3p. This study revealed that the most frequent allelic imbalance is in 1q telomere in both the stroma as well as epithelium of phyllodes tumors and carcinoma of breast, which suggests that in some phyllodes tumors the stroma as well as the epithelium both are neoplastic [117].

Contradictory findings between the epithelial and stromal components in phyllodes tumors in studies (imbalance at D3S1300 in stroma and at D3S1293 in epithelium) has led to doubts as to whether these two components have different clonalities or they arise from one clone but gain different mutations during the process of tumour progression [7]. Most studies have concluded the later to be a more possible one.

Some studies concurred with the finding of gain of 1q but did not reveal any allelic loss at the 3p loci [118]. 3p14 harbours the FHIT (fragile histidine triad) gene, a tumor suppressor gene [118]. The DNA mismatch repair gene homologue (hMLH1) is also present in this location [118]. With the absence of consistent losses in these 3p loci, these abnormalities might not be significant in phyllodes tumors.

Studies have further investigated the genetic imbalances characteristic of phyllodes tumors mostly to analyse whether these will be helpful in evaluating the malignant potential of the tumor[119]. Results revealed that the most frequent gain was found in chromosome 1q as well as chromosomes 5 and 18 [119]. 13q, 6q, 10p and 12q were the common sites where loss of chromosome occurred [119]. Chromosome 13q14.2 has the RB1 gene which is a tumor suppressor that is the most common target for deletion. Gain of chromosome 1q and/or loss of 13q were found to be the trademark alterations in phyllodes tumors. These recurrent chromosome imbalances were found in almost 100% of malignant cases [119]. Analysis of number of chromosome imbalances help in separating benign from borderline tumors and malignant ones [119]. Benign tumors in general showed a median of change in one chromosome. Borderline tumors and malignant tumors on the other hand

showed a median of changes in 6 chromosomes [119]. Statistically there was no difference between borderline tumors and malignant ones [119]. Yet another finding is tumors with few or nil chromosomal imbalances had nuclear size of <50um3, mitotic rate of <3/10hpf, cellularity <100nuclei/1hpf. FISH studies revealed MDM2 and MYC amplifications rarely [119]. MYC amplification has been found in many epithelial tumors [119]. MDM2 has been found to negatively regulate p53. These amplifications along with frequent 13q loss indicates a connection between PTs and sarcomas [119].

While genetic expression forms the basis for oncogenesis, many extracellular factors play a role in the initiation and progression of these tumors. In phyllodes tumors the interaction between the epithelium and the stroma plays an important role.

EPITHELIAL-STROMAL INTERACTIONS

Histopathological features can give clues about epithelial involvement in the stromal proliferation of phyllodes tumor. Perithelial accentuated stromal proliferation is an important clue for this interaction [113]. Increased mitotic activity is seen in the perithelial stroma but not in the stroma distant from the epithelium . This suggests that the epithelium has an influence on stromal growth [120]. The increase in the

perithelial stromal mitotic activity has been proposed to be due the ability of the epithelium to produce a humoral factor, which has a range of action of approximately 200um [120]. The breast epithelium is said to promote estrogen-dependent stimulation of fibroblast DNA synthesis during normal breast development. This is achieved by interaction between the epithelium and stroma, which is mediated by growth factors. A similar interaction probably occurs during tumorigenesis as well.

The Wnt pathway is a cell signal transduction pathway that causes beta-catenin stabilization and translocates it to the nucleus to activate certain genes [121]. Most tumors show stromal nuclear staining by beta-catenin with a periductal accentuation, which suggests the epithelium-dependent proliferation of stromal cells in benign phyllodes tumors [121].Malignant tumors in general show weak or nil staining of stroma [121]. Beta-catenin positivity in the nucleus of stromal cells was found to be associated with expression of epithelial Wnt5a mRNA in excess [121]. This suggests that Wnt5a overexpression in the epithelium can cause stromal growth in phyllodes tumor of benign category [121]. During tumor progression, stromal growth becomes independent of the Wnt pathway [121].

As not all PTs overexpress epithelial Wnt , other possible explanation for the increased stromal expression of beta-catenin was

looked for by studying Insulin-like Growth Factor I and II (IGF I and II) on PTs [122]. IGF-I activates the beta catenin pathway whereas IGF-II translocates beta-catenin to the nucleus [122]. FISH technique has revealed that most phyllodes tumors show widespread stromal overexpression of IGF-II and few cases show stromal overexpression of IGF-I [122]. The latter was associated with beta-catenin positivity in the nucleus of stromal cells and with expression of Wnt5a [122]. This showed that the IGF-I and Wnt signals probably have complementary effects and do not act alternatively to cause beta-catenin overexpression in stromal cell nuclei of PTs [122]. As increased Insulin like Growth Factor expression is found in the stroma away from the epithelium it has been suggested that IGFs may be cause expression of beta-catenin in the stroma distant from epithelium. And WNT5a signal from epithelium could be the reason for expression of beta-catenin in the stroma of subepithelial region[122]. Both Insulin like Growth Factor I and beta-catenin are only minimally expressed in malignant phyllodes, this proves that the pathway does not play a role in stromal overgrowth in malignant PTs and the stromal growth is malignant PTs is autonomous. Stromal beta-catenin positivity in fibroadenomas and PTs tumors shows a link between the two at molecular level [122].

There is inverse association between expression of ER in the epithelium and mitotic count in the stroma. ER shows diminished expression in the borderline and malignant PTs as compared to benign ones [123]. This epithelial expression of ER proves its paracrine action on stromal growth in PTs.

Endothelin 1, a vasoactive peptide has similar role in epithelial stromal interaction.

MALIGNANT PROGRESSION OF PHYLLODES TUMORS

P53 is a tumor suppressor gene present in chromosome 17p13.1[125]. Stromal p53 expression has been consistently reported to increase with phyllodes tumour grade[126], which is represented, by stromal hypercellularity and overgrowth[125]. Strong p53 staining was seen in perithelial stroma in malignant PTs[127]. No correlation has been found with recurrent disease in most studies[125, 126] marked increase in p53 expression was found between benign and malignant tumors [125, 127]. The correlation between p53 epithelial and stromal staining hints about epithelial-stromal interaction in phyllodes tumors.

Ki-67 is a proliferation marker, which is useful in predicting tumor progression in PTs [126]. Assessment of S phase fraction by flow

cytometry shows a marked increase from benign to malignant PTs [126]. And it helpful in predicting prognosis.

Epidermal growth factor receptor (EGFR) [129] is a marker which plays a role in tumor progression via PI3-K/AKT, phospholipase C pathways that play a role in cell motility, adhesion and proliferation [130]. EGFR is seen to be expressed in the stroma of PTs. Staining is seen to progressively increase with increasing tumor grade [129]. EGFR has also been found to be associated with stromal overgrowth, nuclear atypia, mitotic activity, invasive margins and tumor size [129].FISH technique has shown amplifications of EGFR in stroma of PTs and PCR has revealed EGFR amplifications in intron 1 PTs [131]. EGFR overexpression and whole-gene amplifications have not been observed in fibroadenomas [131].

Assessment of microvessel density in stroma of PTs using CD31 has shown marked increase in the number of blood vessels / hpf from benign to malignant PTs. No significant difference exists between borderline phyllodes and malignant phyllodes [135]. VEGF - Vascular endothelial growth factor is a peptide that causes endothelial cell proliferation [136]. VEGF positivity in stroma increases markedly with increase in grade [136]. VEGF activates macrophages that in addition secrete VEGF and some cytokines that promote tumor growth [136].

Heparan sulfate is yet another protein that is believed to be responsible for the invasive and metastatic potential of phyllodes tumors [137]. It is needed for intercellular and extracellular matrix adhesion and is also essential for stabilizing growth factor binding (fibroblast growth factor to their receptors) [137].10E4 antibody detect heparan sulfate in tissues. Perithelial stroma and basement membrane showed strong expression in approximately 10% of PTs [137]. Strong 10E4 expression was seen in stroma of high-grade phyllodes tumors [137].

No association has been found between 10E4 stromal positivity and individual histological parameters in different grades of PTs[137]. Heparan sulfate is now considered as one of the molecules contributing to proliferation of stroma in PTs. CD10 - CALLA (common acute lymphoblastic leukemia antigen) is a member of a family of metalloproteases that also shows increased stromal expression with increasing grade in PTs [137].

Marked increases in c-kit expression in the stroma from benign to malignant phyllodes is seen [136] it has been localized to subepithelial stroma [132]. The c-kit is hence an important contributor to stromal growth in PTs [125,126,132,133]. It is presumed to participate in cell cycle progression. There is a significant correlation between p53 immuno staining and c-kit expression[125]. c-kit expression in stroma shows significant correlation

with grade of phyllodes tumor and recurrence [125]. c-kit was also been found to show moderate to strong positivity in the epithelial component of benign phyllodes tumors in contrast with the negative staining of epithelial component of the malignant ones [133] implying autocrine/paracrine activation and interdependence of stroma and epithelium.

CD34 is predominantly expressed in stroma of benign phyllodes tumors [134]. On the other hand only few malignant tumors showed stromal staining [134]. There is inverse correlation between CD34 expression and actin expression which demonstrates myofibroblastic differentiation in most malignant tumors and only few benign ones [134].

CD117

- Proto-oncogene
- Also known as c-kit , stem cell factor receptor
- Gene at 4q11-21
- It's the receptor for kit protein- a 145 kD tyrosine kinase growth factor receptor protein which is important for development and survival of mast cells, hematopoietic stem cells, melanocytes, germ cells, interstitial cells of Cajal

- It has activating or gain of function mutations most often at exon 11 and less often at exons 9 and 13. Tyrosine kinase activity of c-kit has been found to be inhibited by Imatinib mesylate (Gleevec, STI571) a tyrosine kinase inhibitor used to treat c-kit positive tumors.

INTERPRETATION

Should be strong and diffuse cytoplasmic staining, like the positive control.

Investigation of CD117 status in phyllodes tumors first begun in the year 2000. Two point mutations Q556X and N564S involving the juxtamembrane domain, which is exon 11, were found. Mutations of this domain affect the autoinhibitory action of the receptor and can lead to activation of the receptor even when there is no ligand binding to it. However some studies revealed no such mutations neither in exon 11 nor in exons 9, 13, and 17 which are other commonly reported regions of mutations. Several other studies have reported scant findings of silent mutations or mutations of unknown significance. One study has revealed a point mutation of L510M in exon 10, which is of unknown significance. It has also been reported that a silent mutation of isoleucine 798 in exon 17 is also involved in a few cases.

Despite the lack of activating mutations involving the *KIT* gene, overexpression of CD117 has been reported in phyllodes tumors. Many reports have shown an association between CD117 protein expression and increasing grade with increased expression of CD117 being noted in the malignant tumors. Toluidine blue staining is routinely performed to rule out the possibility of the confounding effect of mast cells, as it has been suggested that the associations observed might be caused by mast cell phenomenon. Toluidine blue has been found to be negative in cases that are CD117 positive.

The variable results obtained from different groups can be because of the variable antibodies, different staining protocols and scoring criteria that are used. There is no universal agreement currently achieved as to which protocol and scoring criteria are best suited. Standardization of protocols in the various laboratories has been a challenging task and all the antibodies used have to be optimized and validated individually. **For scoring criteria 1% cutoff is used because of the fact that CD117 is not expressed normally in breast stromal cells [23] and even a low percentage of CD117 expression can be an indicator of an abnormal state**. This is exemplified by most of the studies done so far in which, CD117 positivity noted on immunohistochemistry showed only a low percentage of positive cells.

It has been observed that CD117-positive cases show a shorter period of recurrence free survival. This correlates with other study findings that showed association between CD117 stromal positivity and tumor recurrence. Some studies have shown a higher percentage of CD117-positive tumors among cases that showed metastasis.

A significant poor survival outcome has also been observed in patients with tumors showing stromal CD117-positivity. These findings point to a poorer clinical outcome among tumors expressing CD117, which suggests that these tumors are of aggressive nature.

Investigations about CD117 protein mutation and expression were largely motivated by the successful use of tyrosine kinase inhibitors in patients with Gastro Intestinal Stromal Tumors. It has been suggested that the stromal component of phyllodes tumors and Gastro Intestinal Stromal Tumors have some similarities like their spindle cell nature and their spectrum of behavior from benign to malignant. Recent insights into the roles played by CD117 in cancer have shed light on the different types of tumors expressing CD117. On a broader scale CD117-expressing tumors have been classified into two main categories: (1) those having activating (gain-of-function) CD117 mutations and derived from cells that are normally found to express CD117. In such cases CD117 has got a central pathogenetic role in the initiation of neoplasm

(2) those in which CD117 mutations occur only rarely, here tumors are made of cells that normally do not express CD117. CD117 has got a passive role in these neoplasms and its expression is acquired only during the process of tumor progression. This explains the lack of mutations seen in phyllodes tumors in comparison to GISTs. GISTs arise due to neoplastic transformation of interstitial cells of Cajal that are normally found to express high levels of CD117. On a comparative note the stromal component of phyllodes tumors has been found to arise from breast mesenchymal tissue which, does not usually express CD117 under the normal circumstances.

CD117 protein expression correlates with borderline/malignant tumors and with worse pathologic parameters. Immunohistochemically CD117 positivity may be seen in tumors without the presence of activating mutations.

CD34

CD34 is a type I transmembrane glycoprotein expressed in hemopoietic stem cells, endothelial cells, some fibroblast and bone marrow progenitor cells and is expressed in many mesenchymal tumors.

Terminology

- It is also called hematopoietic progenitor cell antigen CD34
- CD34+ stromal cells are called dendritic interstitial cells

Pathophysiology

- Intercellular adhesion protein and cell surface glycoprotein and the ligand is CD62L (L-selectin)
- Mediates attachment of hematopoietic stem cells to bone marrow extracellular matrix or to stromal cells
- CD34 staining defines adult hematopoietic stem cells but CD34+ cells can also differentiate into neural cells

CD34 is a transmembrane glycoprotein which is believed to be involved in modulation of signal transduction and cell adhesion and it is expressed by mesenchymal cells at various sites including the stroma of breast. Loss of CD34 in mesenchymal cells has been found in several cases where there is malignant transformation of mesenchymal cells. Malignant phyllodes tumours of breast have been found to exhibit lower levels of CD34 expression than the benign tumors.

"Loss of CD34 has been correlated with invasive potential".

CD34 expression is persistently lost in malignant phyllodes tumors. Loss of expression is also seen in invasive ductal carcinoma, some cases of ADH but not around glandular structures showing LCIS. This is to be noted because ADH and DCIS are considered to be premalignant lesions on the other hand LCIS is said to confer an increased risk for the development of carcinoma yet the risk relates to the development of carcinoma in both breasts and not to the site of the LCIS.[40] This suggests that loss of CD34 may have correlation with invasive potential. The loss of CD34 expression has been found to be very localised with loss being seen predominantly around ducts showing DCIS changes with expression around adjacent normal breast glands being retained. This strongly implicates the epithelial–mesenchymal interactions in the control of expression of this marker. Which factors determine the loss of CD34 is of interest because not all cases show loss and this points to different functional states of the tumor cells. Alterations in the stroma seen in malignancy are increased hyaluronic acid ,increased expression of ED-A fibronectin and vascular endothelial growth factor.

MATERIALS & METHODS

MATERIALS AND METHODS

STUDY DESIGN

The present study is a retroprospective study. Retrospective study period is june 2014-june 2015 and prospective study period is july 2015-july 2016.

Ethical clearance for the study was obtained from the Ethics Committee of Coimbatore Medical College, Coimbatore.

A total sample of 25 cases of phyllodes tumor of breast were analyzed.

PLACE OF STUDY

The study is undertaken in the Department of Pathology, Coimbatore Medical College and hospital, Coimbatore

STUDY PERIOD

JUNE 2014-JULY2016

SELECTION CRITERIA

(a) Inclusion Criteria

- Breast lumpectomy/mastectomy specimens diagnosed as phyllodes tumor histopathologically
- Age group: 20-70years

(b) Exclusion Criteria

- Overfixed specimens
- specimen not sent in formalin/ill fixed specimens

The study includes mastectomy/lumpectomy specimens received from surgery and surgical oncology departments and diagnosed as phyllodes tumor histopathologically . Specimens fixed in 10% formalin, routinely processed and embedded in paraffin blocks , sectioned at 5 microns thickness and stained with haematoxylin and eosin were taken, then histological grading of tumor was assessed based on stromal cellularity, nuclear pleomorphism, stromal overgrowth, mitotic rate, margin of tumor. The threshold for number of mitoses required for classification into each subgroup were <2 mitoses/10 HPFs for benign PTs, 2-5 mitoses/10 HPFs for borderline PT, and >5 mitoses/10 HPFs for malignant PTs. Stromal overgrowth has been taken as marked

stromal proliferation to the point where the epithelial component is absent in at least 1 low-power field (×40). Immunohistochemical staining was used for demonstration of CD117 and CD34.

IHC SCORING CRITERIA FOR CD 117

Staining pattern of CD 117 is both cytoplasmic and membranous. Intensity of staining of stromal cells for CD 117 will be assessed using cytoplasmic staining of breast epithelium as internal control.

Staining is graded based on intensity of staining and percentage of spindle cells that took up the stain.

Scoring criteria 1% cutoff is generally used because of the fact that CD117 is not expressed normally in breast stromal cells and even a low percentage of the protein expression can be an indicator of an abnormal state[150].

INTERPRETATION OF CD 34 EXPRESSION

- Membranous positivity
- Endothelium acts as positive internal control
- Staining is graded based on intensity of staining and percentage of stained spindle cells.
- CD34-positive tumors were defined as those with more than 10% of the tumor cells staining positive for CD34[142].

REAGENTS USED IN IMMUNOHISTOCHEMISTRY

1. Peroxide- block
2. Power- block
3. Chromogen - Diaminobenzidine
4. DAB substrate-liquid
5. Super- enhancer
6. Poly HRP reagent
7. Hematoxylin which acts as counter stain
8. various buffer solutions

BUFFERS USED

1. **TRIS EDTA : pH- 9.0**

   TRIS buffer salt - 6.05 grams

   Disodium EDTA- 0.744 grams

   Distilled water - 1000ml

2. **TRIS BUFFER pH - 8**

   TRIS buffer salt - 6.05 grams

   Sodium chloride -8 grams

   Distilled water - 1000ml

   1N Hydrochloric acid - 3 ml

3. **CITRATE BUFFER pH-6**

   Trisodium citrate - 2.94 grams

   Distilled water : 1000ml

   1N Hydrochloric acid : 5 ml

IMMUNOHISTOCHEMISTRY PROCEDURE

1. Incubate the slides overnight in incubator at 60^0C
2. Deparaffinise the tissue sections in xylene for 30 minutes
3. Wash using absolute alcohol for five minutes - two changes
4. Wash with tap water for ten minutes
5. Rinse using distilled water for five minutes
6. Antigen retrieval - done by placing the slides in a microwave

4. with appropriate buffers for 20 minutes

7. Cool it in room temperature and then rinse in distilled water
8. Washing is done in TBS buffer for five minutes - two changes
9. Apply peroxide block for ten minutes
10. Washing is done in TBS buffer for five minutes - two changes
11. Power block is applied on sections for ten minutes
12. Drain the slide and add primary antibody which is followed by incupation at room temperature in a moisture chamber for 1 hour
13. TBS buffer wash for five minutes - two changes.
14. Slides are covered with superenhancer for thirty minutes
15. Washing is done in TBS buffer for five minutes - two changes

16. Reagent of poly HRP is applied for thirty minutes.
17. Washing is done in TBS buffer for five minutes - two changes.
18. DAB chromogen is applied for five to eight minutes.
19. Washing is done in TBS buffer for five minutes - two changes.
20. Tap water wash is done for five minutes.
21. Counterstaining is done with Mayers hematoxylin for one minute.
22. Tap water wash is done for five minutes.
23. Air dried and mounted in DPX.

STATISTICAL ANALYSIS

Statistical correlation between stromal expression of CD34, CD117 and histopathological grade were analysed using Chi square test. p values of less than 0.05 were taken as significant.

Statistical Analysis:
The data are reported as the mean +/- SD or the median, depending on their distribution.
Frequencies are expressed in percentages.
The differences in quantitative variables between groups were assessed by means of the un paired t test. Comparsion between groups was made by the Non parameteric Mann - whitney test
Comparison between groups were assesed by ANOVA
The chi square test was used assess differences in categoric variables between groups.
A p value of <0.05 using a two-tailed test was taken as being of significance for all statistical tests. All data were analysed with a statistical software package .(SPSS, version 16.0 for windows)

OBSERVATIONS AND RESULTS

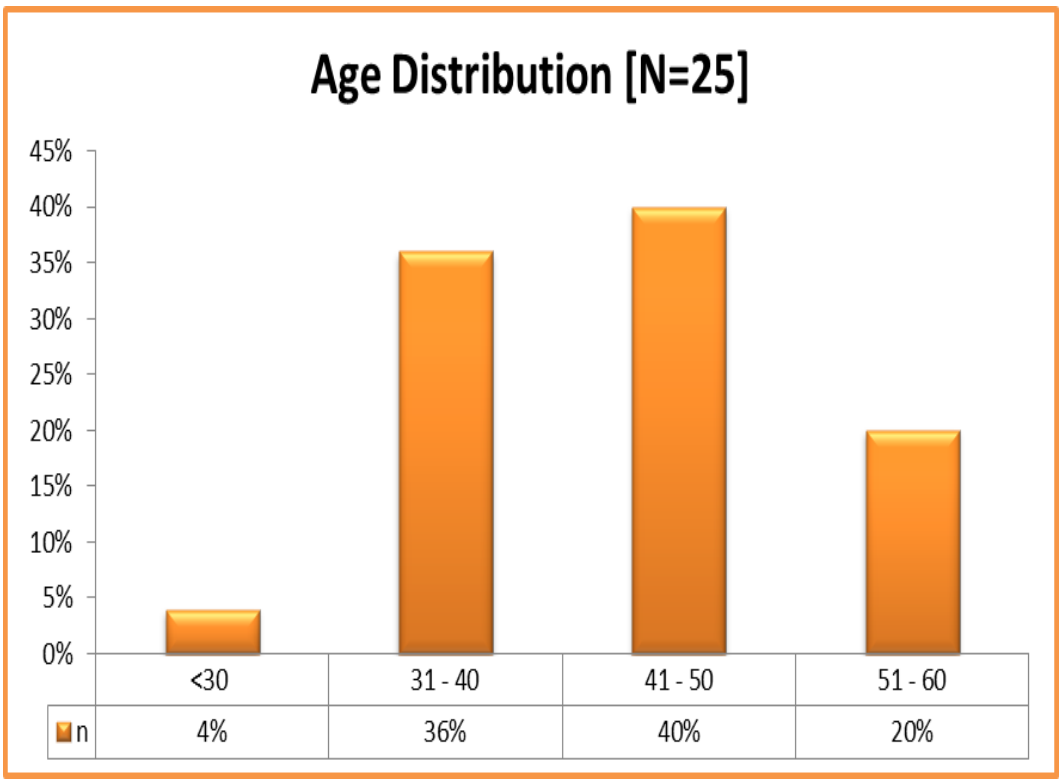
OBSERVATION AND RESULTS

TABLE.1 DISTRIBUTION OF PHYLLODES TUMOR OF BREAST ACCORDING TO DIFFERENT AGE GROUP

Age Distribution		
Age Group	n	(%)
<30	1	4%
31 - 40	9	36%
41 - 50	10	40%
51 - 60	5	20%
Total	**25**	**100%**

Most of the tumors around (76%) belonged to the age group between 30 and 50 years.

CHART.1 DISTRIBUTION OF PHYLLODES TUMOR OF BREAST ACCORDING TO DIFFERENT AGE GROUP



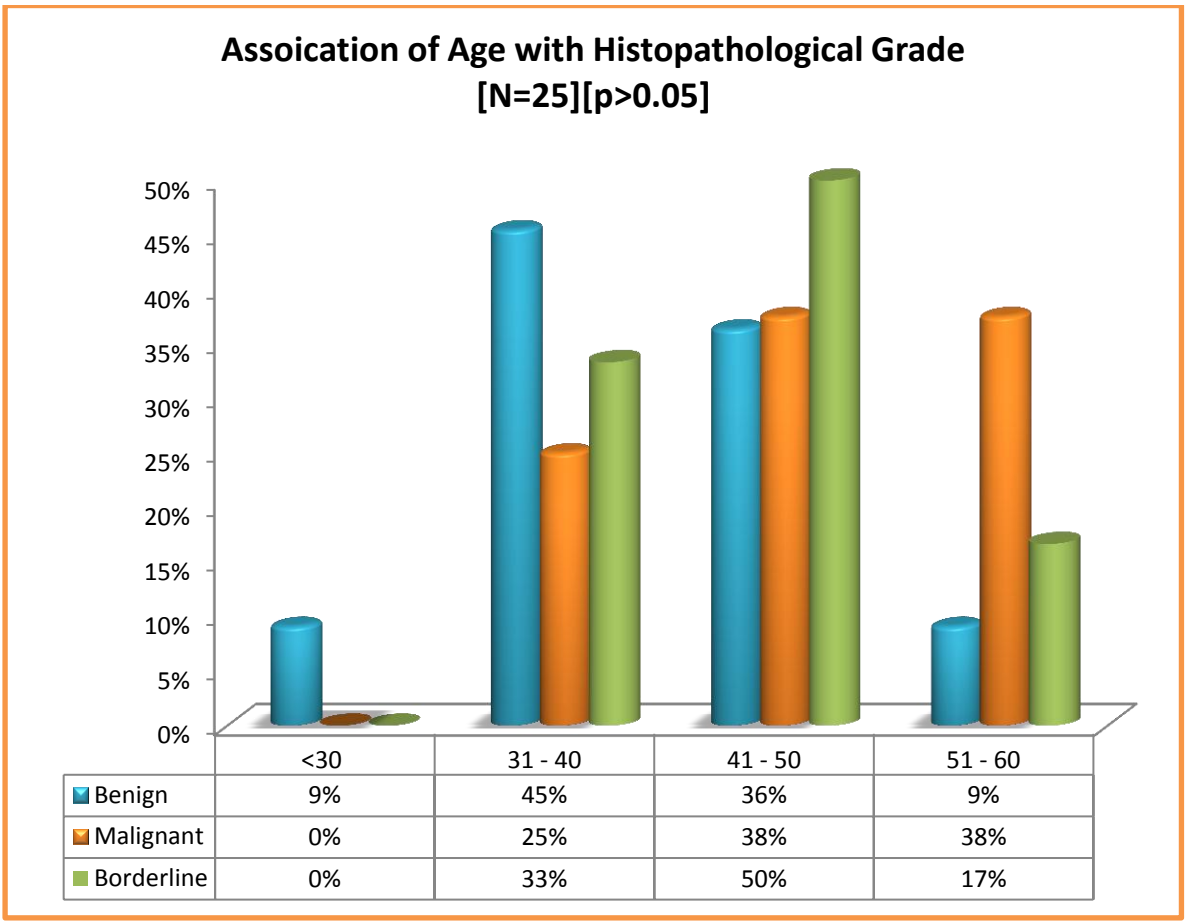
Most of the tumors around (76%) belonged to the age group between 30 and 50 years.

TABLE 2: ASSOCIATION OF AGE WITH HISTOPATHOLOGICAL GRADE

Association of Age with Histopathological Grade				
	GRADE			
Age	**Benign**	**Malignant**	**Borderline**	**Total**
<30	1	0	0	1
31 - 40	5	2	2	9
41 - 50	4	3	3	10
51 - 60	1	3	1	5
TOTAL	**11**	**8**	**6**	**25**

Most of the benign tumors belonged to the age group between 30 and 50 years(80%) and the majority of malignant tumors belonged to the age group between 40 and 60 years(76%)

CHART 2: ASSOCIATION OF AGE WITH HISTOPATHOLOGICAL GRADE



	<30	31 - 40	41 - 50	51 - 60
Benign	9%	45%	36%	9%
Malignant	0%	25%	38%	38%
Borderline	0%	33%	50%	17%

Most of the benign tumors belonged to the age group between 30 and 50 years(80%) and the majority of malignant tumors belonged to the age group between 40 and 60 years(76%)

TABLE 3: MEAN AGE OF OCCURRENCE OF TUMOR IN DIFFERENT HISTOPATHOLOGICAL CATEGORIES

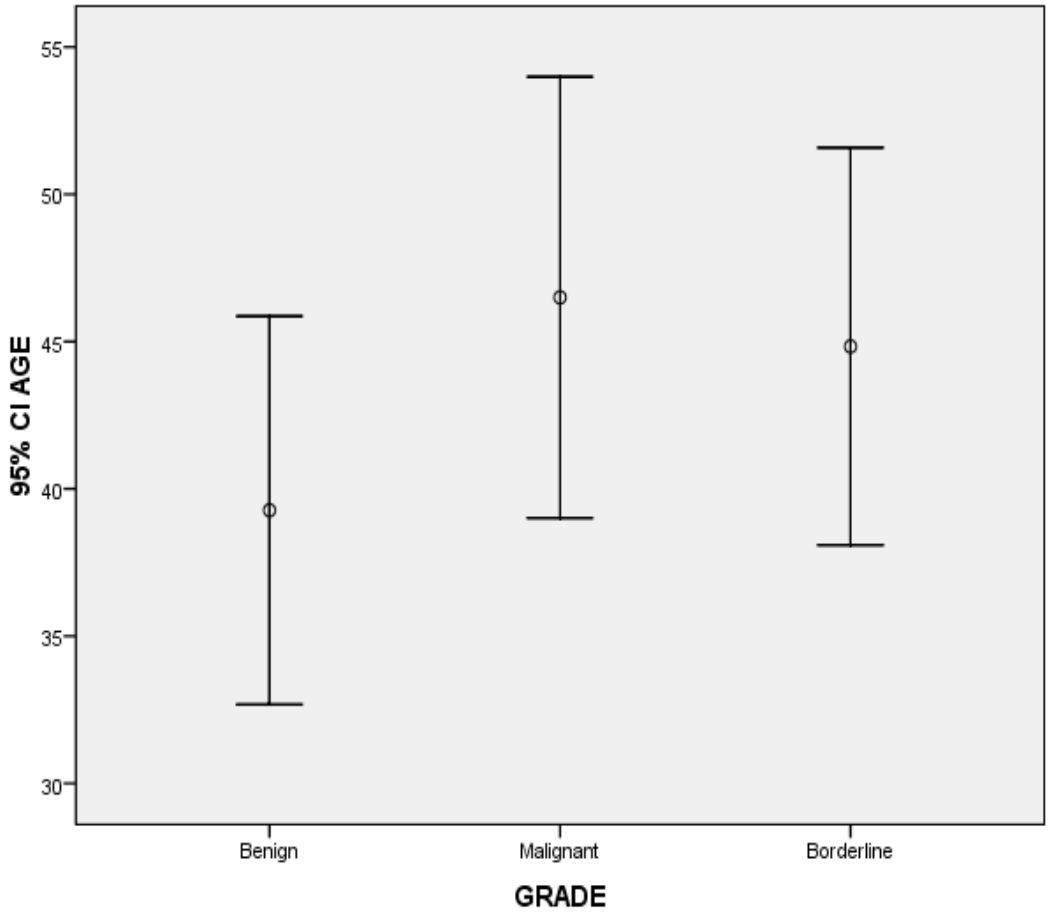
Mean Age with Histopathological Grade							
	95% CI for Mean						
	Mean	**SD**	**Lower**	**Upper**	**Minimum**	**Maximum**	**Sig**
Benign	39.27	9.809	32.68	45.86	20	58	
Malignant	46.5	8.96	39.01	53.99	33	56	
Borderline	44.83	6.432	38.08	51.58	39	57	>0.05
Total	**42.92**	**9.133**	**39.15**	**46.69**	**20**	**58**	

Mean age for benign tumors-39.27

Mean age for borderline tumors-44.83

Mean age for malignant tumors-46.5

CHART 3: MEAN AGE OF OCCURRENCE OF TUMOR IN DIFFERENT HISTOPATHOLOGICAL CATEGORIES



Mean age for benign tumors-39.27

Mean age for borderline tumors-44.83

Mean age for malignant tumors-46.5

TABLE 4: PERCENTAGE OF TUMORS IN EACH HISTOPATHOLOGICAL GRADE

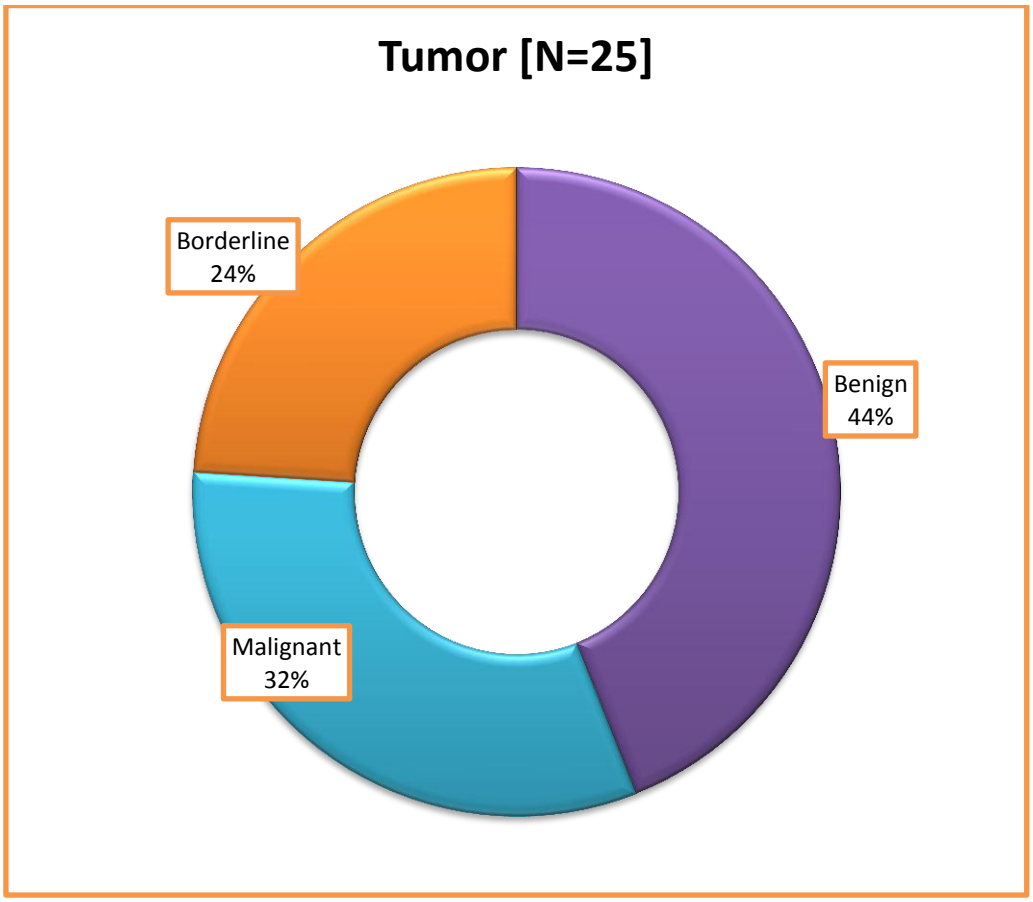
Prevalence of Tumor		
Grade	**n**	**(%)**
Benign	11	44%
Malignant	8	32%
Borderline	6	24%
Total	**25**	**100%**

Overall percentage of benign tumors-44%

Overall percentage of borderline tumors-24%

Overall percentage of malignant tumors-32%

CHART 4: PERCENTAGE OF TUMORS IN EACH HISTOPATHOLOGICAL GRADE



Overall percentage of benign tumors-44%

Overall percentage of borderline tumors-24%

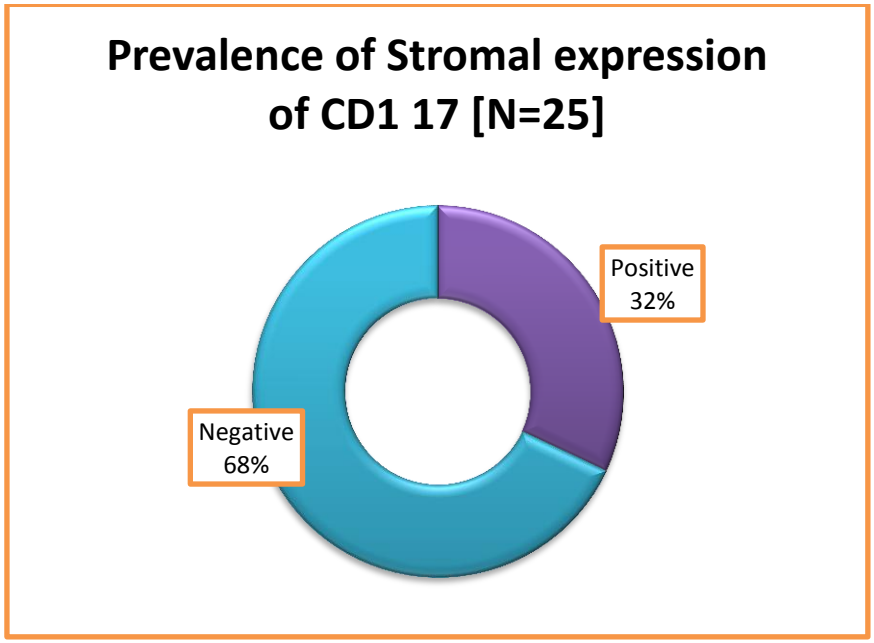
Overall percentage of malignant tumors-32%

TABLE 5: OVERALL STROMAL EXPRESSION OF CD117

OVERALL STROMAL EXPRESSION OF CD117		
CD 117	**n**	**(%)**
Positive	8	32%
Negative	17	68%
Total	**25**	**100%**

Overall 32% of cases(8/25) showed stromal positivity for CD117.

CHART 5: OVERALL STROMAL EXPRESSION OF CD117



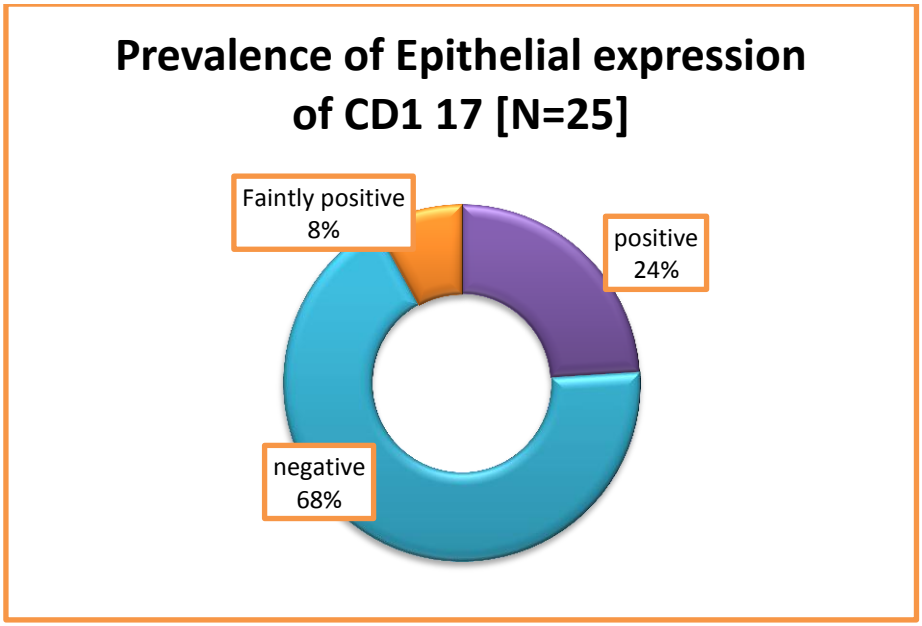
Overall 32% of cases 8/25 showed stromal positivity for CD117.

TABLE 6: OVERALL EPITHELIAL EXPRESSION OF CD117

OVERALL EPITHELIAL EXPRESSION OF CD117		
Expression	**n**	**(%)**
positive	6	24%
negative	17	68%
Faintly positive	2	8%
Total	**25**	**100%**

Overall 32% of cases 8/25 showed epithelial positivity for CD117

CHART 6: OVERALL EPITHELIAL EXPRESSION OF CD117



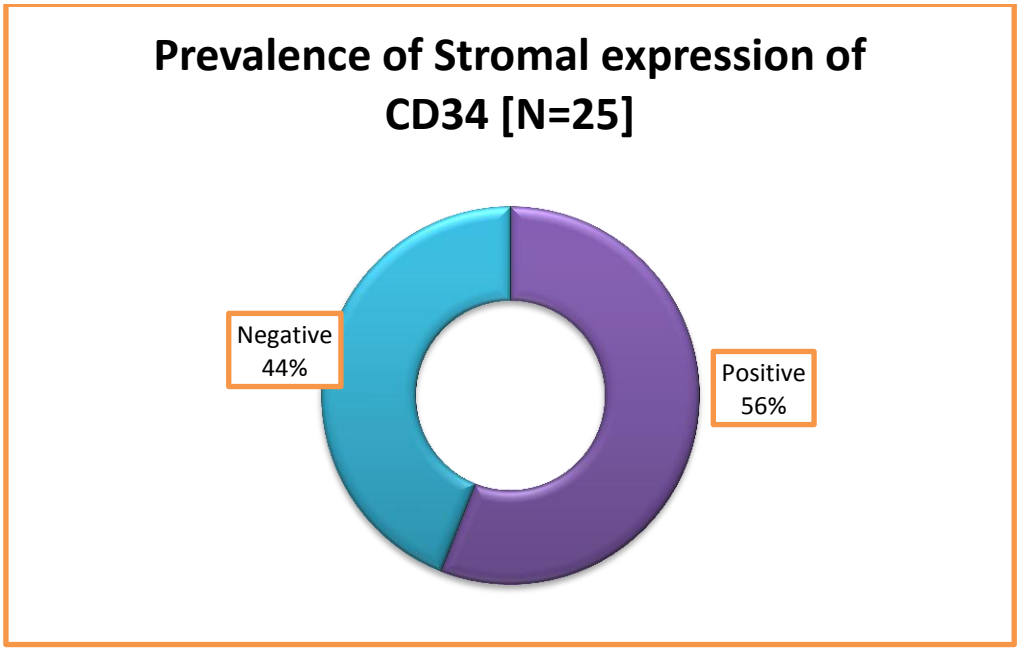
Overall 32% of cases (8/25) showed epithelial positivity for CD117

TABLE 7: OVERALL STROMAL EXPRESSION OF CD 34

OVERALL STROMAL EXPRESSION OF CD34		
Expression	n	(%)
Positive	14	56%
Negative	11	44%
Total	25	100%

Overall 56% of tumors (14/25) showed stroma positivity for CD34.

CHART 7: OVERALL STROMAL EXPRESSION OF CD34



Overall 56% of tumors (14/25) showed stroma positivity for CD34.

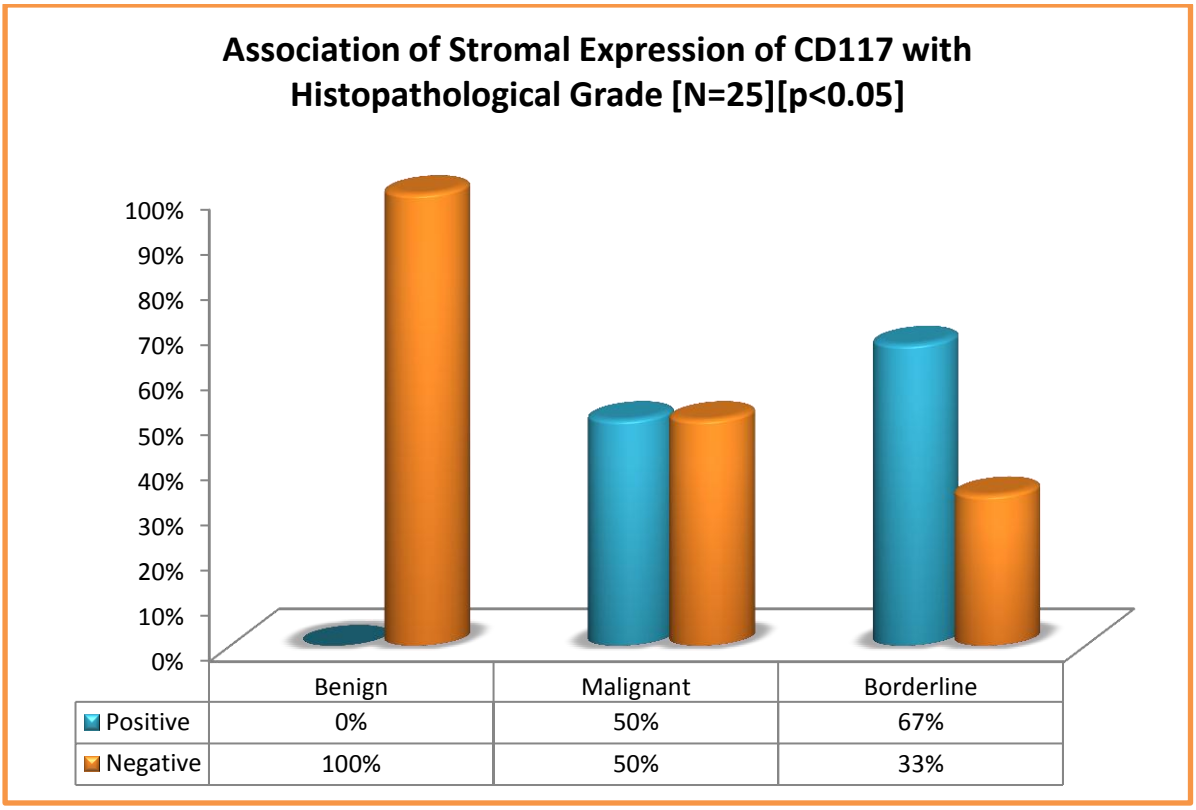
TABLE 8: ASSOCIATION OF STROMAL EXPRESSION OF CD117 WITH HISTOPATHOLOGICAL GRADE

Association of Stromal expression of CD117 in Phyllodes Tumor with Histopathological Grade			
	CD117		
Grade	**Positive**	**Negative**	**Total**
Benign	0	11	11
Malignant	4	4	8
Borderline	4	2	6
TOTAL	8	17	25

Out of the 8 cases that showed positivity for CD117, 4 cases were malignant and 4 were borderline.

None of the benign cases showed stromal positivity for CD117.

CHART 8: ASSOCIATION OF STROMAL EXPRESSION OF CD117 WITH HISTOPATHOLOGICAL GRADE



Overall 50% of malignant tumors(4/8 cases) showed stromal positivity for CD117 and 67% of borderline tumors showed stromal expression of CD117. None of the benign cases showed stromal positivity for CD117(0%).

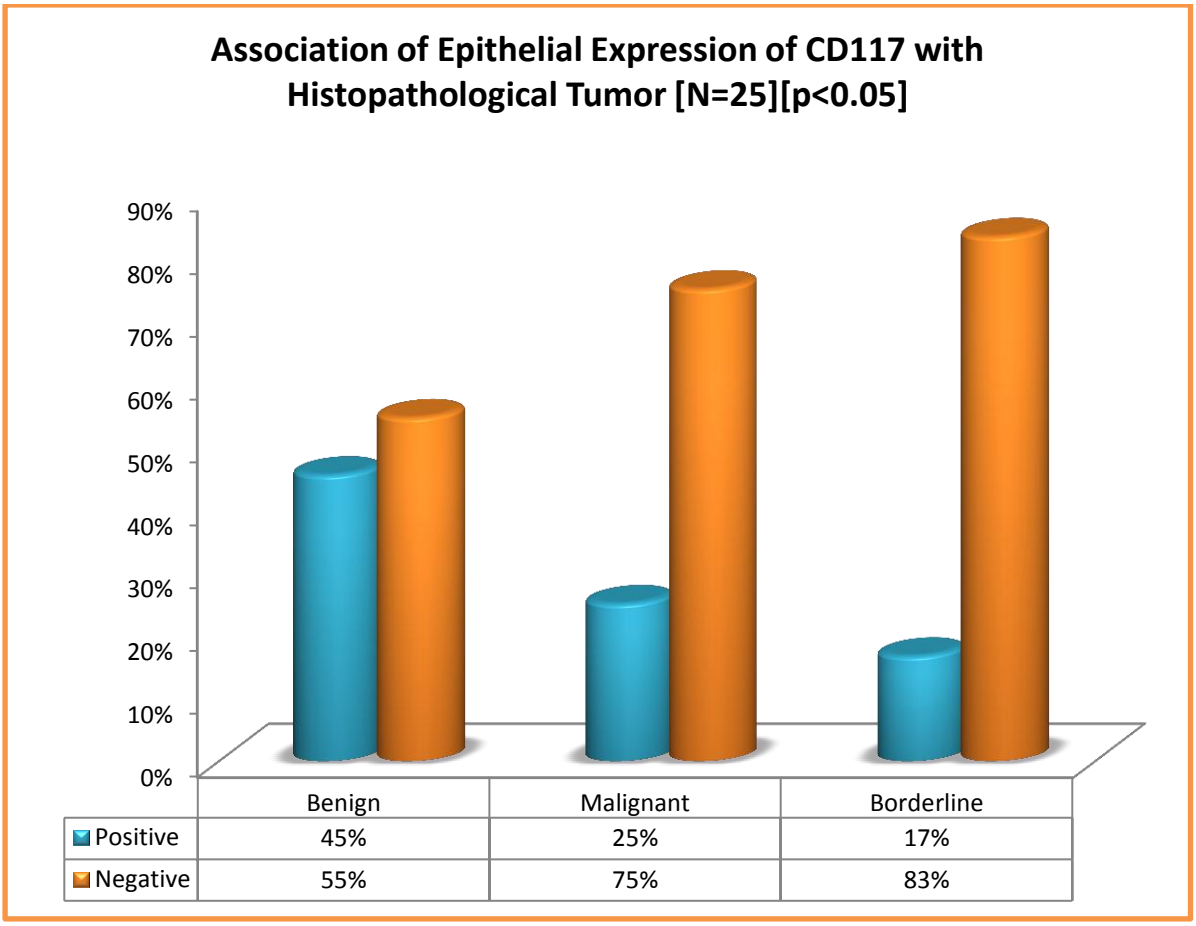
The association of stromal expression of CD117 with malignant and borderline tumors is statistically significant, p value is less than 0.05 (Chi- square test).

TABLE 9 :ASSOCIATION OF EPITHELIAL EXPRESSION OF CD117 WITH HISTOPATHOLOGICAL GRADE

Association of Epithelial Expression of CD117 with Histopathological Grade			
	Epithelial		
Grade	**Positive**	**Negative**	**Total**
Benign	5	6	11
Malignant	2	6	8
Borderline	1	5	6
TOTAL	8	17	25

5 out of 11 benign tumors showed epithelial positivity for CD117. Only 2 out of 8 malignant tumors and 1 out of 6 borderline tumors showed epithelial expression of CD117.

CHART 9: ASSOCIATION OF EPITHELIAL EXPRESSION OF CD117 WITH HISTOPATHOLOGICAL GRADE



Overall 45% of benign tumors(5/11 cases) showed epithelial positivity for CD1117. Only 17% of borderline tumors and 25% of malignant tumors showed epithelial expression of CD117.

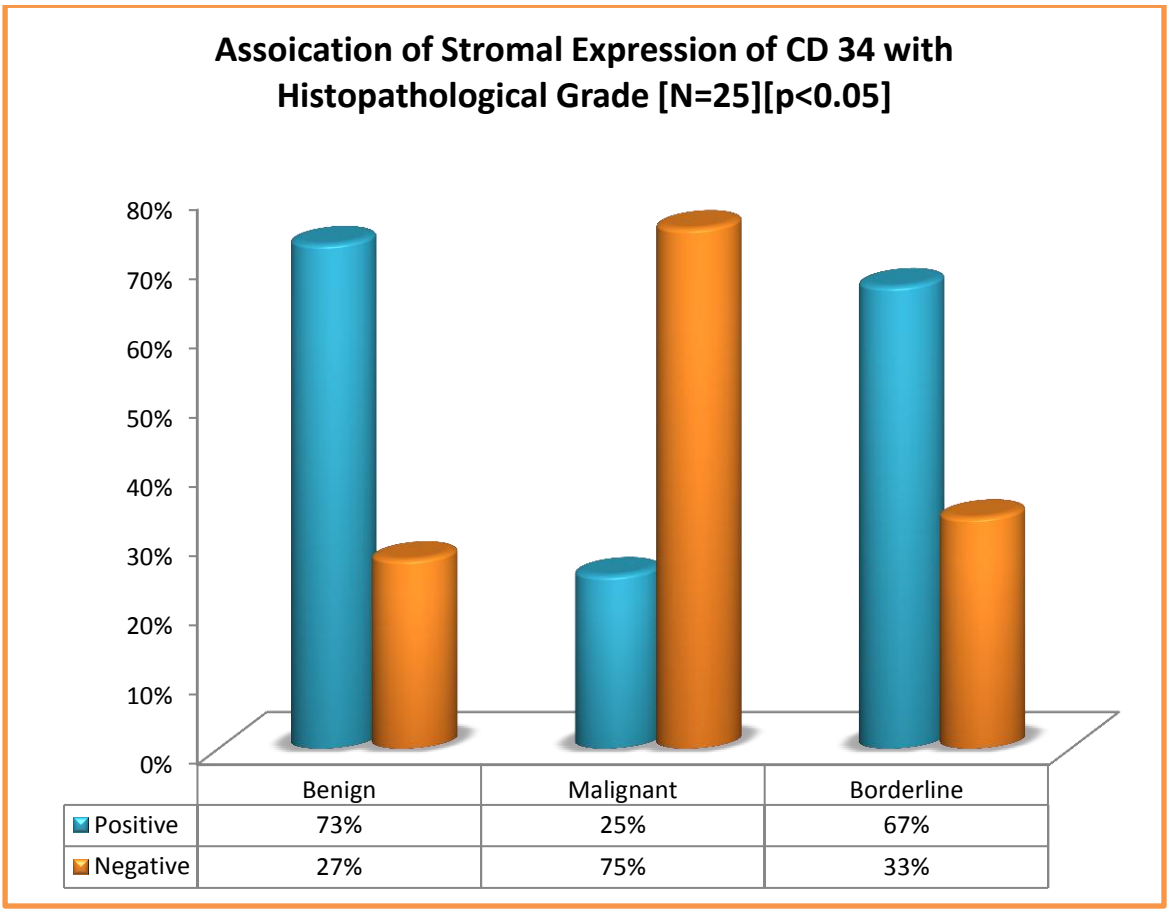
The association of epithelial expression of CD117 with benign tumors is statistically significant, p value is less than 0.05 (Chi- square test).

TABLE 10: ASSOCIATION OF STROMAL EXPRESSION OF CD34 WITH HISTOPATHOLOGICAL GRADE

Association of Stromal Expression of CD34 in Phyllodes tumor with Histopathological Grade			
	CD 34		
Grade	**Positive**	**Negative**	**Total**
Benign	8	3	11
Malignant	2	6	8
Borderline	4	2	6
TOTAL	14	11	25

Out of the 14 cases that showed positivity for CD34, 8 cases were benign and 4 were borderline and 2 were malignant.

CHART 10: ASSOCIATION OF STROMAL EXPRESSION OF CD34 WITH HISTOPATHOLOGICAL GRADE



Overall 73% of benign tumors(8/14 cases) showed stromal positivity for CD34. 67% of borderline tumors (4/6) and 25% of malignant tumors showed stromal expression of CD34.

The association of stromal expression of CD34 with benign tumors and borderline tumors is statistically significant, p value is less than 0.05 (Chi- square test).

TABLE 11: FREQUENCY OF CD34/CD117 IMMUNOPROFILES IN THE 3 MORPHOLOGICAL CATEGORIES OF PHYLLODES TUMORS

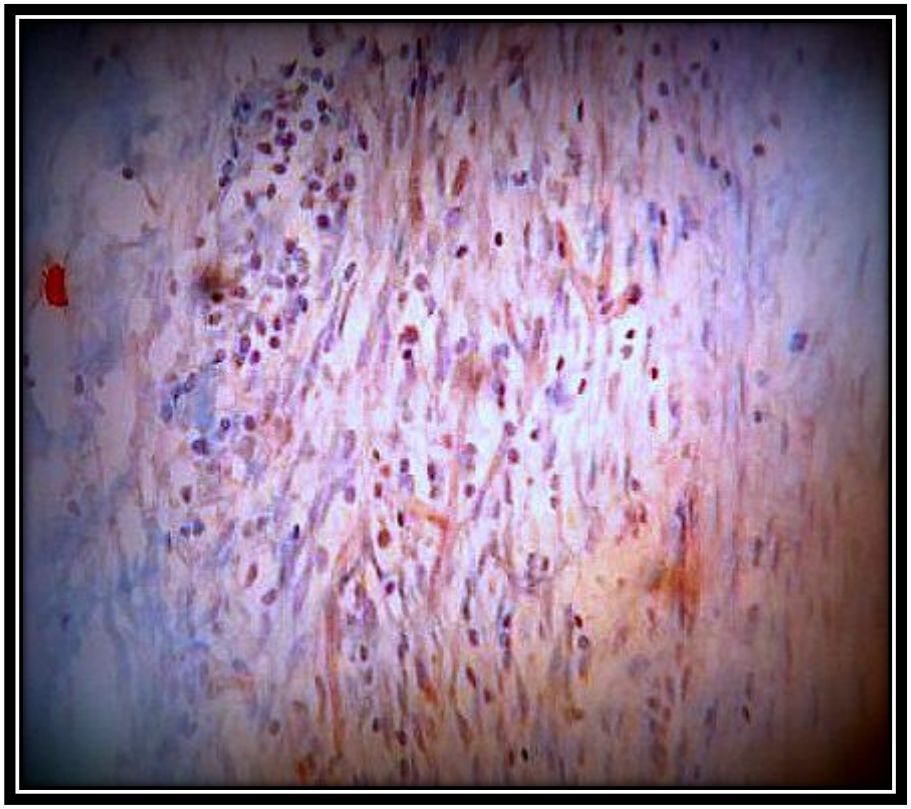
Histopathological grade	CD34+ / CD117-	CD34-/ CD117+	CD34+/ CD117+	CD34-/ CD117-
BENIGN	8/11(73%)	0/11(0%)	0/11(0%)	3/11(27%)
BORDERLINE	1/6(17%)	1/6(17%)	3/6(50%)	1/6(17%)
MALIGNANT	2/8(25%)	4/8(50%)	0/8(0%)	2/8(25%)

Most malignant PTs (50%) showed a CD34-/CD117+ immunohistochemical profile, whereas most benign PTs commonly showed the CD34+/ CD117- immunoprofile (73%).The borderline PTs commonly coexpressed both markers (50%).

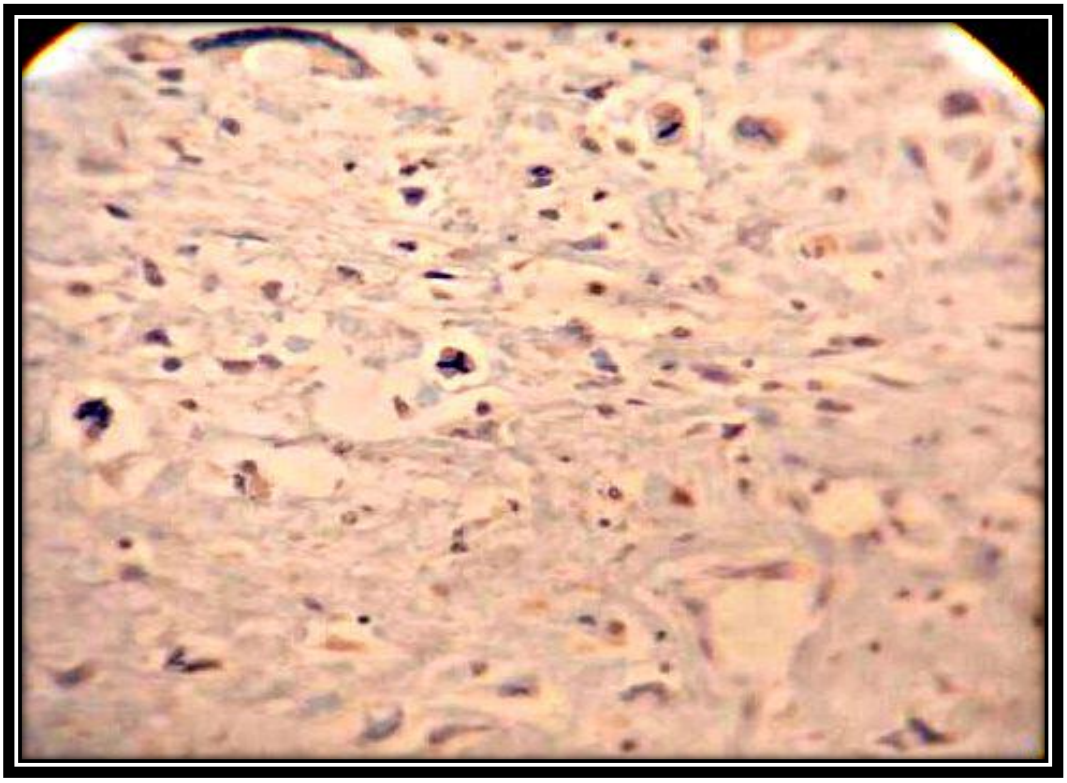
COLOUR PLATES

COLOUR PLATES

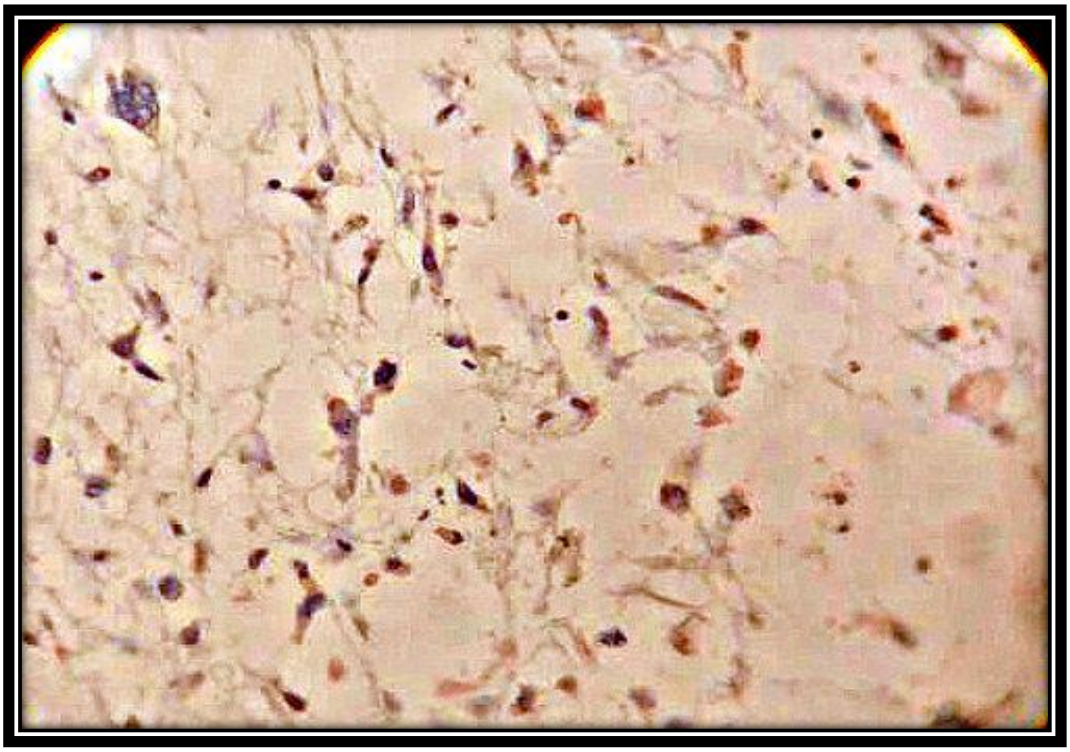
COLOUR PLATE 1:
IMMUNOHISTOCHEMISTRY OF STROMAL CD117 IN MALIGNANT PHYLLODES TUMOR



PICTURE SHOWING CD117 STROMAL POSITIVITY (40X)



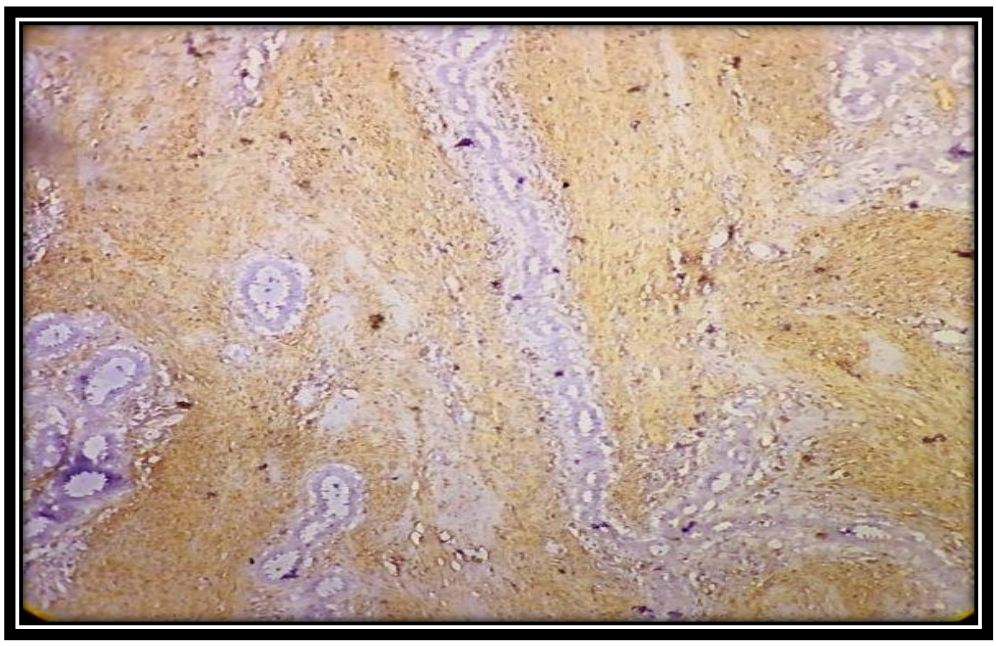
PICTURE SHOWING CD117 STROMAL POSITIVITY IN MALIGNANTPHYLLODES (40X)



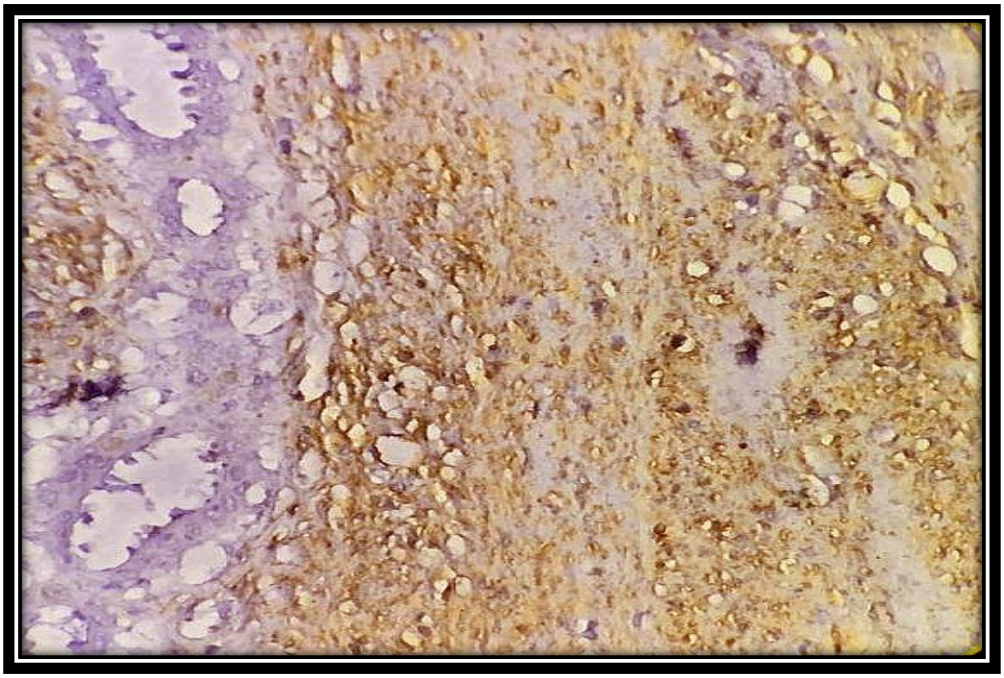
PICTURE SHOWING CD117 STROMAL POSITIVITY IN MALIGNANT PHYLLODES (40X)

COLOUR PLATE2:

IMMUNOHISTOCHEMISTRY OF STROMAL CD34 IN BENIGN PHYLLODES TUMOR



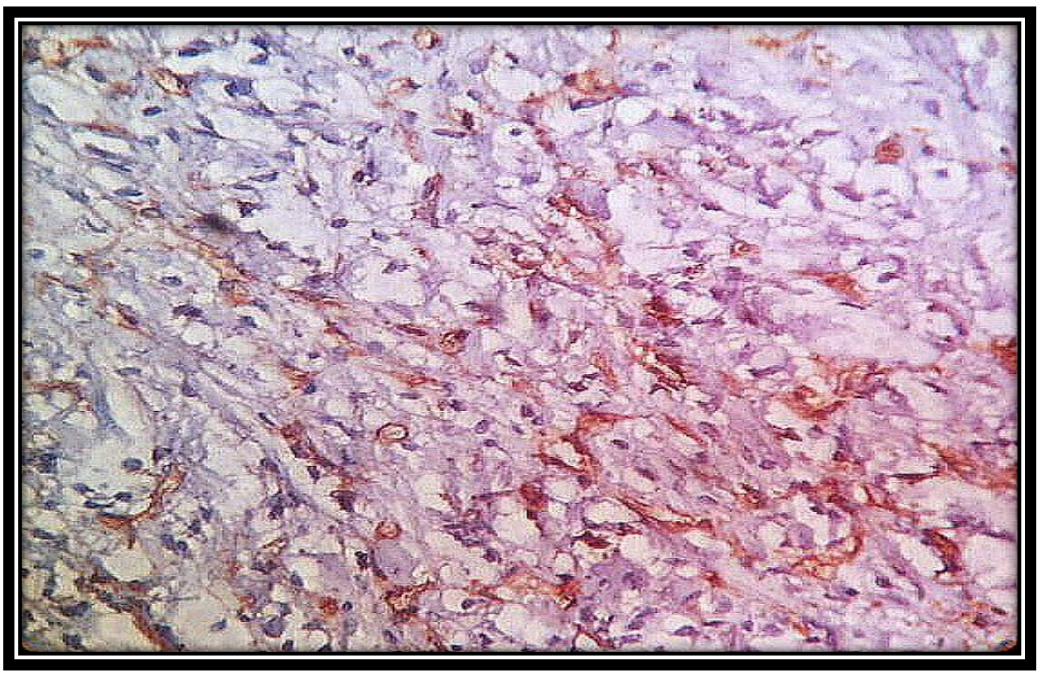
PICTURE SHOWING STROMAL CD 34 POSITIVITY IN BENIGN PHYLLODES (10X)



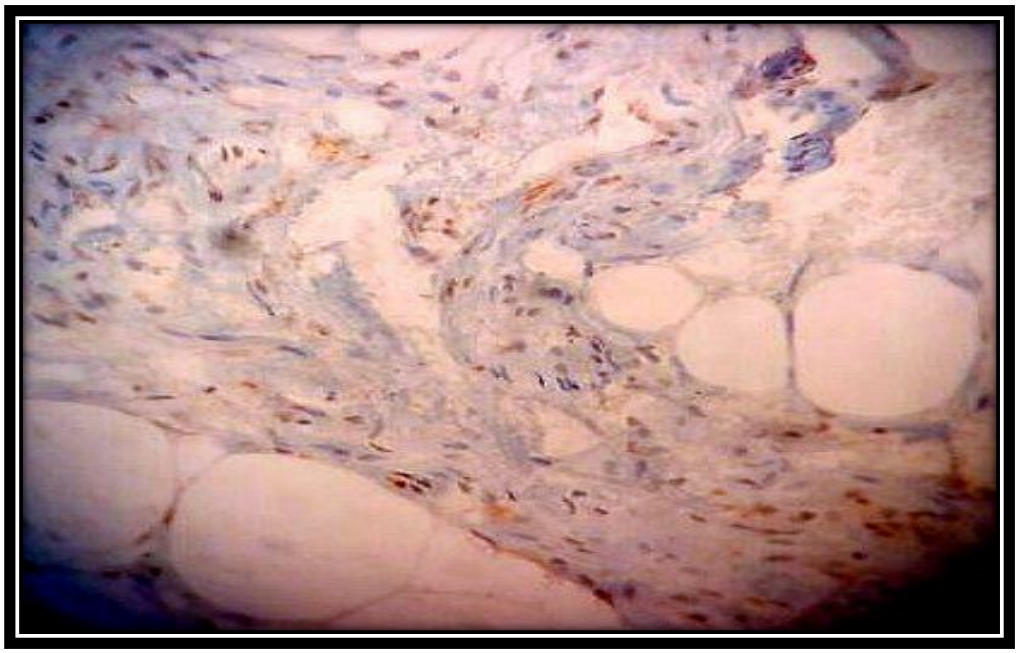
PICTURE SHOWING STROMAL CD 34 POSITIVITY IN BENIGN PHYLLODES (40X)

COLOUR PLATE 3:

IMMUNOHISTOCHEMISTRY OF STROMAL CD34 AND CD117 IN BORDERLINE PHYLLODES TUMOR



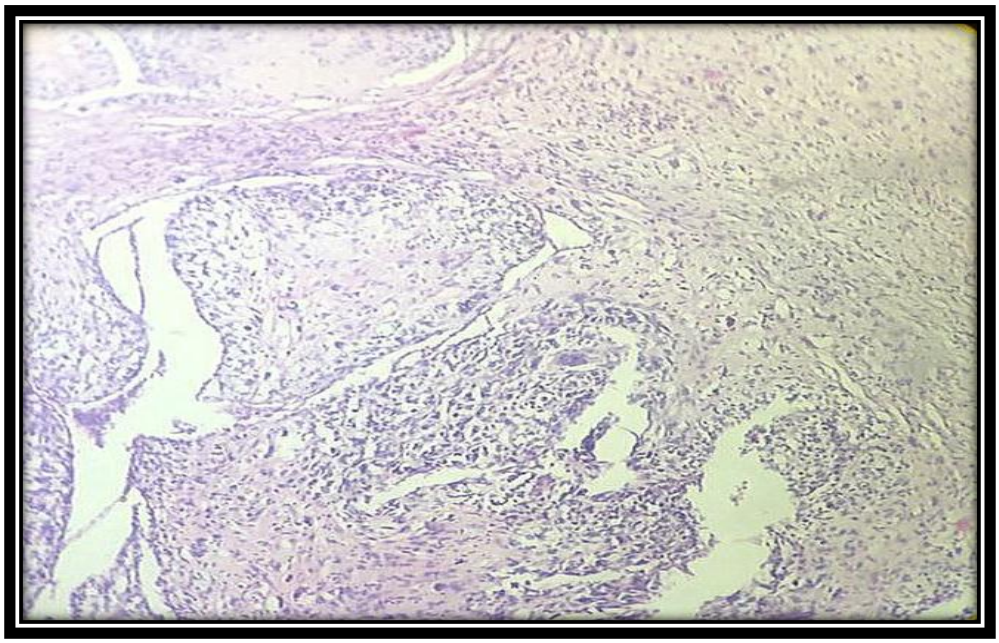
PICTURE SHOWING CD34 STROMAL POSITIVITY IN A BORDERLINE PHYLLODES TUMOR(40X)



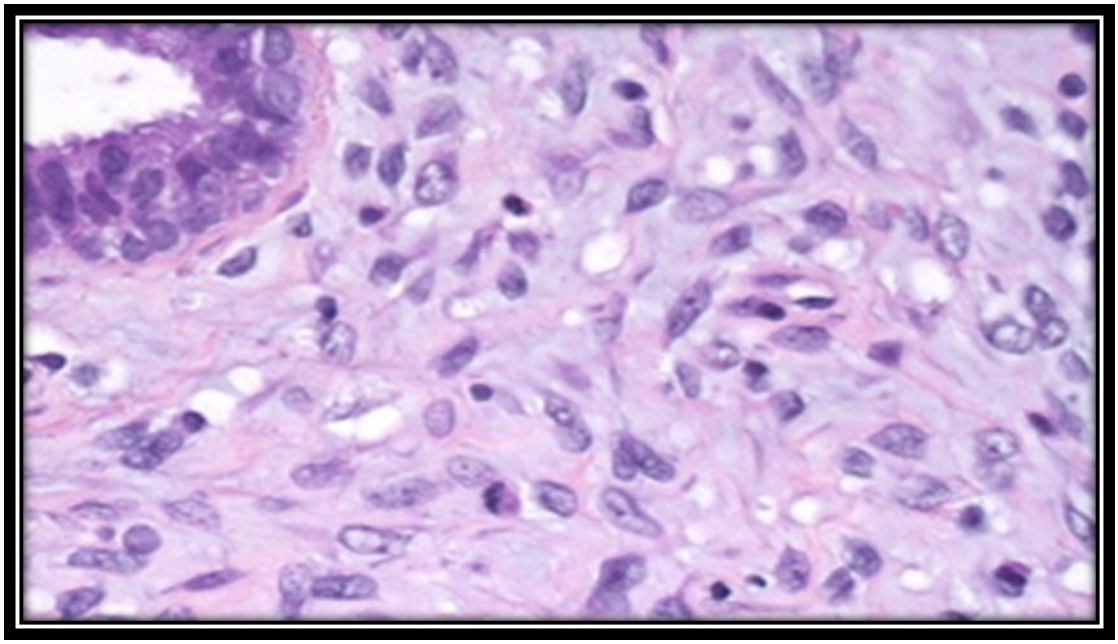
PICTURE SHOWING CD117 STROMAL POSITIVITY IN A BORDERLINE PHYLLODES TUMOR(10X)

COLOUR PLATE 4:

HEMATOXYLIN AND EOSIN STAINING OF MALIGNANT PHYLLODES TUMOR



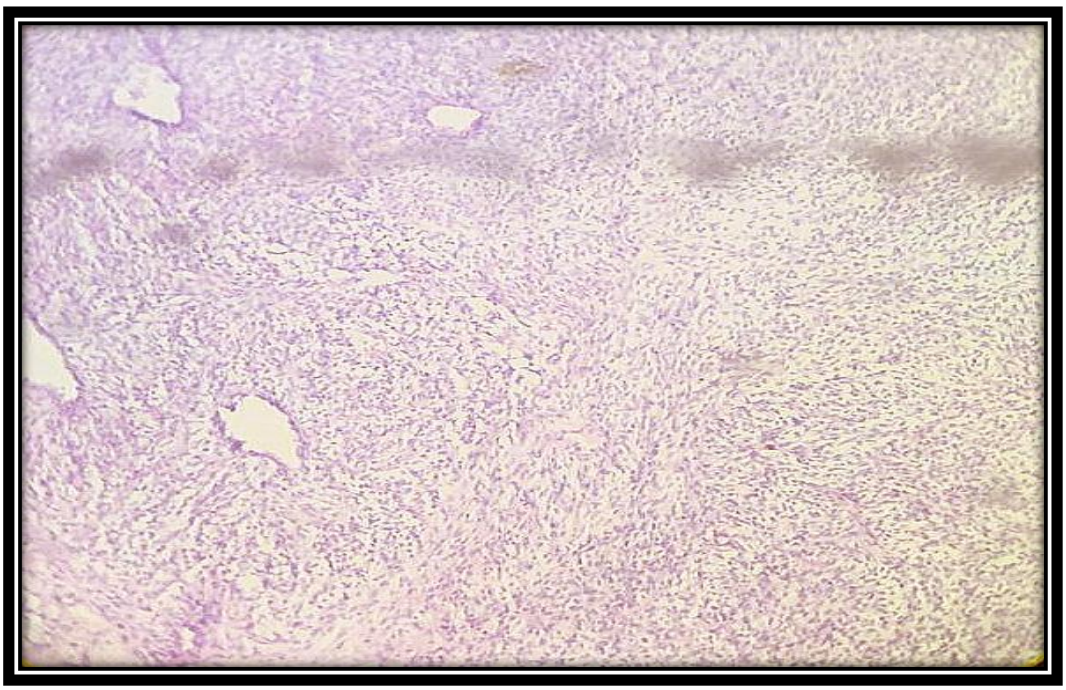
H&E PICTURE OF LOW GRADE MALIGNANT PHYLLODES TUMOR(10X)



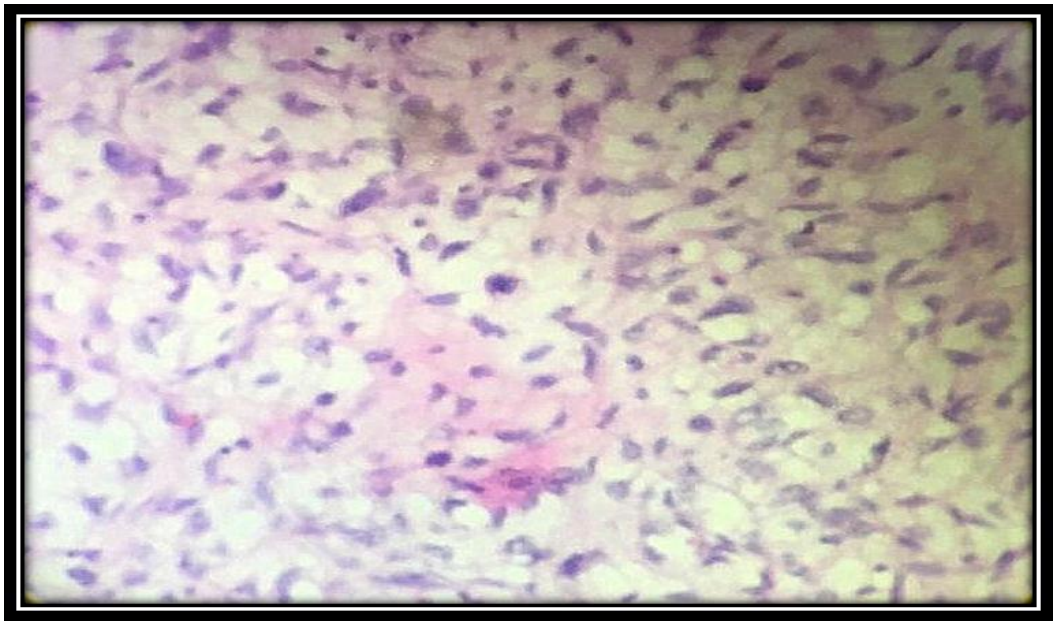
H&E PICTURE OF HIGH GRADE MALIGNANT PHYLLODES TUMOR(40X)

COLOUR PLATE 5:

HEMATOXYLIN AND EOSIN STAINING OF BORDERLINE PHYLLODES TUMOR



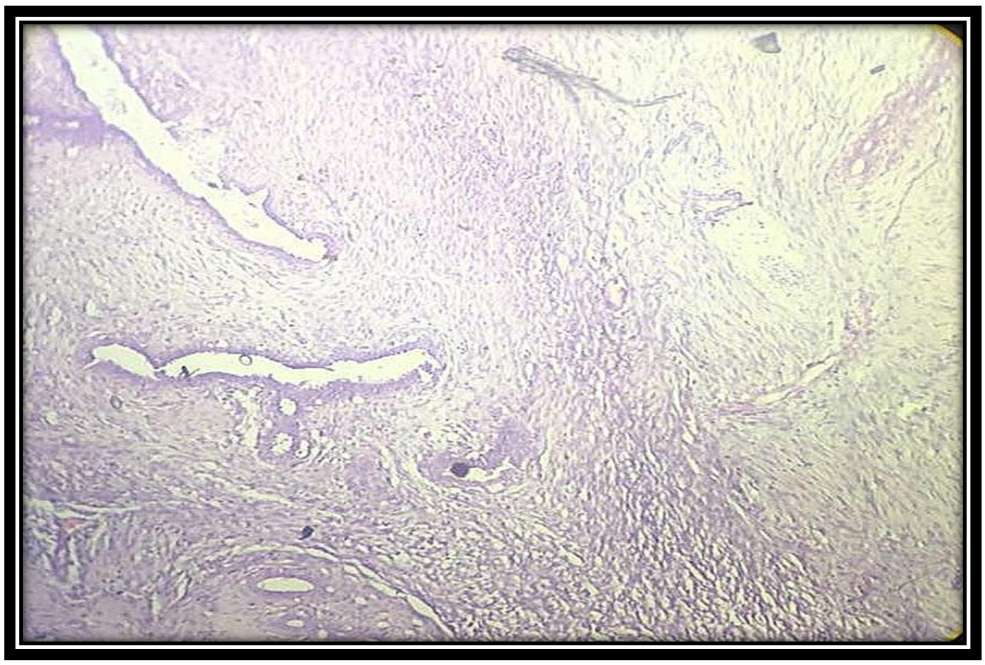
PICTURE SHOWING H&E STAINING OF BORDERLINE PHYLLODES TUMOR(10X)



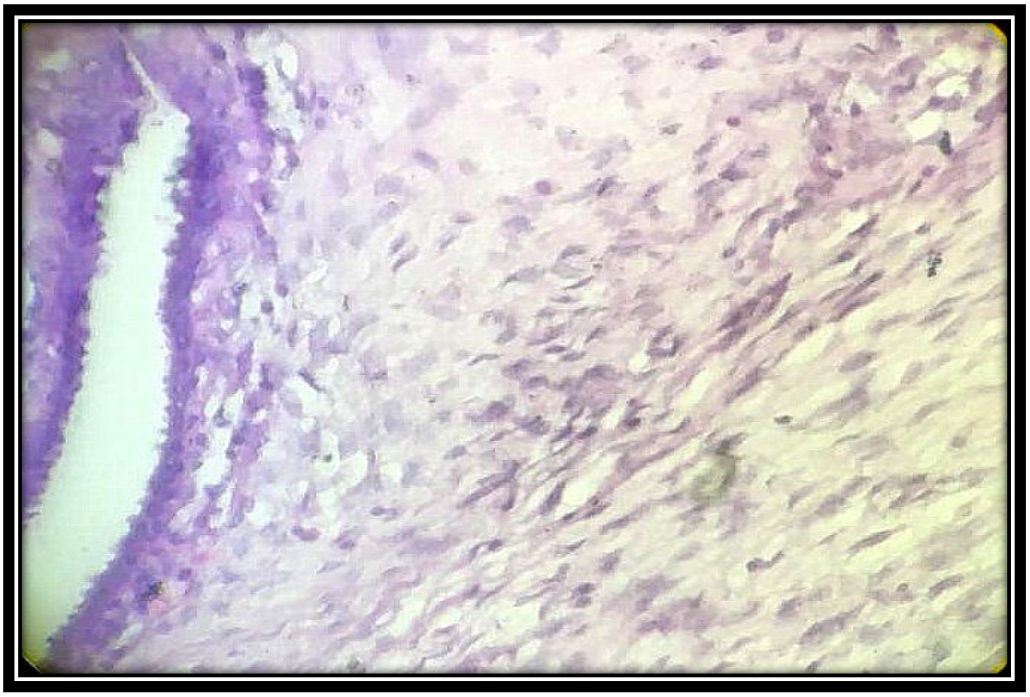
PICTURE SHOWING H&E STAINING OF BORDERLINE PHYLLODES TUMOR(40X)

COLOUR PALATE 6:

HEMATOXYLIN AND EOSIN STAINING OF BENIGN PHYLLODES TUMOR



PICTURE SHOWING H&E STAINING OF BENIGN PHYLLODES TUMOR(10X)



PICTURE SHOWING H&E STAINING OF BENIGN PHYLLODES TUMOR(40X)

COLOUR PLATE 7

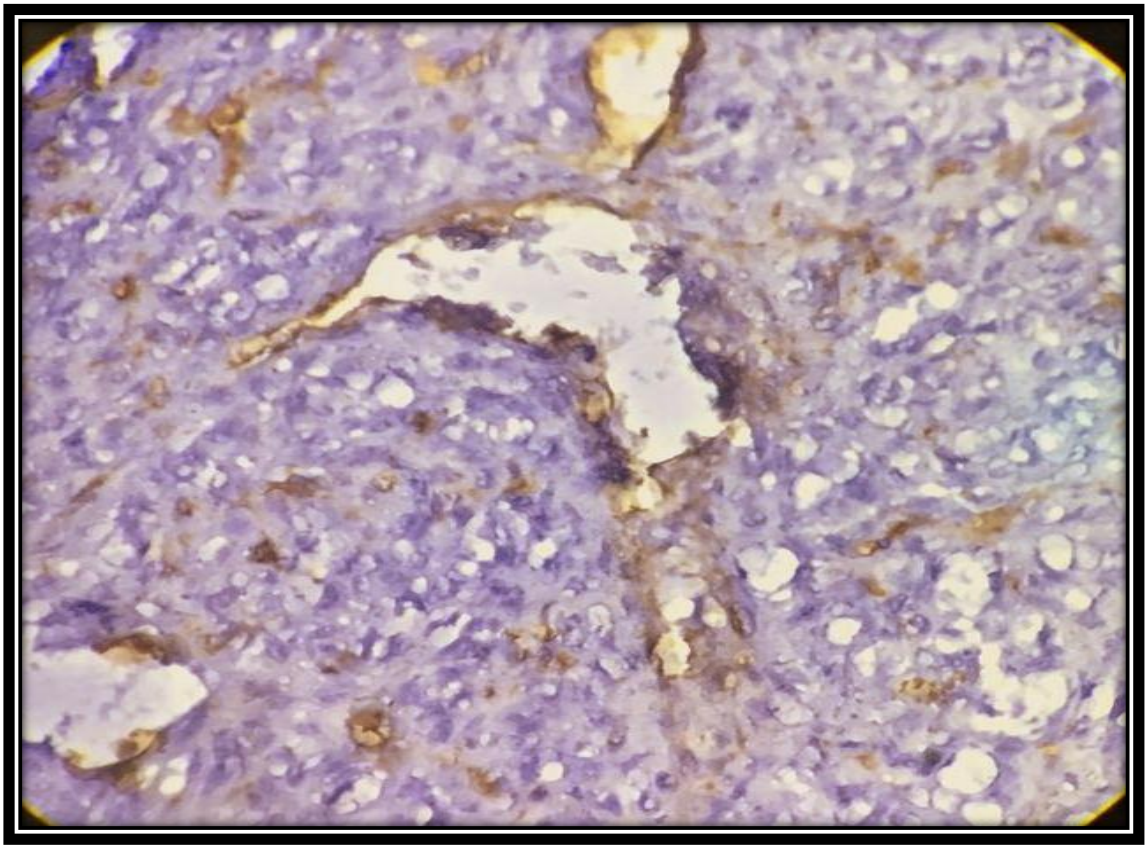
IMMUNOHISTOCHEMISTRY OF EPITHELIAL CD117 IN BENIGN PHYLLODES TUMOR

PICTURE SHOWING EPITHELIAL CD117 POSITIVITY IN BENIGN PHYLLODES(10X)

COLOUR PLATE 8

IMMUNOHISTOCHEMISTRY OF VASCULAR CD34

POSITIVITY IN MALIGNANT PHYLLODES



PICTURE SHOWING INCREASED VASCULARITY AND CD 34

POSITIVITY OF VESSELS IN MALIGNANT PHYLLODES(40X)

DISCUSSION

DISCUSSION

Among the tumors reported in the Department of Pathology of Coimbatore Medical College phyllodes tumor accounts for 0.5 percentage.

It accounts for 6.25% percentage of breast tumors reported in the Department of Pathology of Coimbatore Medical College

In the present study phyllodes tumors were found to occur in the age groups between 20 and 60 years. Most of the benign cases belonged to the age group between 30 and 50 years and malignant cases belonged to the age group between 40 and 60 years.

The mean age of occurrence of phyllodes tumor was 43 years.

The mean age of occurrence of benign phyllodes tumors was 39 years and for malignant phyllodes tumors it was 46 years.

Histological grading of phyllodes tumor in our study was done based on stromal cellularity, nuclear pleomorphism, stromal overgrowth, mitotic rate, margin of tumor.

CD117, or c-kit is a proto-oncogene that encodes a tyrosine kinase receptor.

Various studies have shown increased expression CD117 in the stroma of malignant PTs. In keeping with the findings of other studies our study also showed increased expression of CD117 in the stroma of malignant PTs. The results of various the various studies have been summarized and compared with our findings in the table that follows

Study	**No. of cases**	**Benign phyllodes tumors**	**Borderline phyllodes tumors**	**Malignant phyllodes tumors**
Chen et al[142]	19	1/7 (14.3%)	NA	9/12 (75%)
Sawyer et al[143]	30	1/20 (5%)	NA	5/10 (50%)
Tse et al[144]	179	17/101 (17%)	12/50 (24%)	13/28 (46%)
Carvalho et al[145]	19	6/13 (46.2%)	NA	6/6 (100%)
Tan et al[146]	273	7/206 (3.4%)	4/41(9.8%)	6/26(23.1%)
Esposito et al[147]	30	2/16 (13%)	5/8 (63%)	4/6 (67%)
Noronha et al[148]	33	7/21 (33.3%)	4/6(66.7%)	6/6 (100%)
Our study	25	0/11(0%)	4/6(66.7%)	4/8(50%)

The association of stromal expression of CD117 with malignant tumors is statistically significant, p value is less than 0.05

Normal stromal cells do not express CD117 and even 1% positivity is significant[150]. In our study the percentage of stromal cells that showed positivity for CD117 ranged from 1%-30% .

CD34 is a transmembrane sialomucin glycoprotein.

Our study showed CD34 expression in 8/11 cases (73%) of benign PTs, 4/6 cases (66.7%) of borderline PTs, and 0/8 cases (0%) of malignant PT.

Silverman and Tamsen in their study showed that CD34 is expressed stroma of benign phyllodes tumors[149]. Chen et al in their study of 19 cases of PT found that CD34 was preferentially expressed in benign PTs[142]. Noronha et al also in their study of 33 cases found that CD34 had a predominant stromal expression in benign phyllodes tumors[148]. Their results are similar to our study and are summarized and compared with our findings in the following table

Study	No.of cases	Benign phyllodes tumor	Borderline phyllodes tumor	Malignant phyllodes tumor
Chen et al[142]	19	6/7 (85.7%)	NA	3/12 (25%)
Noronha et al[148]	33	18/21 (85.7%)	6/6 (100%)	1/6 (16.7%)
Our study	25	8/11(73%)	4/6(67%)	2/8(25%)

The association of stromal expression of CD34 with benign tumors is statistically significant,

p value is less than 0.05

In our study, in addition we found that CD117 was expressed in the epithelium and myoepithelium of 5/11 (45%)benign phyllodes tumors, 1/6(17%) borderline tumors and 2/8(25%) of malignant tumors. So CD117 seems to be preferentially expressed in the epithelium of benign phyllodes tumors as well.

The association of epithelial expression of CD117 with benign tumors is statistically significant, p value is less than 0.05.

We also found that as the grade of the tumor increased, the vascularity also increased which was indicated by higher number of CD34 expressing vessels in malignant tumors. 5/8 (62.5%)of malignant tumors showed increased vascularity as indicated by increased number of CD34 positive vessels.

In our study most malignant PTs (50%) showed a CD34-/CD117+ immunohistochemical profile whereas most benign PTs on the other hand commonly showed the CD34+/ CD117- immunoprofile (73%).The borderline PTs commonly coexpressed both markers (50%).

Noronha et al study also showed similar findings wherein most of the malignant PTs (83.3%) showed a CD34-/CD117+ immunoprofile, benign PTs most commonly showed the CD34+/ CD117- immunoprofile (52.4%) and the borderline PTs most commonly coexpressed both markers (66.7%).

SUMMARY

SUMMARY

A study conducted at Coimbatore Medical College, Coimbatore .

Study period being june 2014-july 2016. The study title is "**STUDY OF EXPRESSION OF CD 117 AND CD 34 IN PHYLLODES TUMOR OF BREAST AND ITS CORRELATION WITH HISTOPATHOLOGICAL GRADE"**

The study consists of 25 cases of phyllodes tumor of breast. In all the cases immunohistochemistry was done with markers CD34 and CD117.

Histological Grading of phyllodes tumor was done using stromal cellularity, nuclear pleomorphism, stromal overgrowth, mitotic rate, margin of tumor.

Statistical analysis was done and results were compared with various available previous studies.

This study showed

1. Majority of benign phyllodes tumors belonged to the age group of 30-50 years
2. Majority of malignant phyllodes tumors belonged to the age group of 40-60 years

3. The mean age of occurrence of phyllodes tumor was 43 years.

4. The mean age of occurrence of benign phyllodes tumor in this study was 39 years

5. The mean age of occurrence of malignant phyllodes tumor in this study was 46 years

6. Overall 32% (8 out of 25) of the cases showed positivity for CD117 in the stroma

7. Stromal CD117 positivity was found in 50% cases of malignant Phyllodes tumors of breast. Association of CD117 expression with malignant phyllodes tumor of breast is statistically significant. p value is < 0.05

8. Overall 48% (12 out of 25) of the cases showed positivity for CD34 in the stroma

9. Stromal CD34 positivity was found in 73% cases of benign Phyllodes tumors of breast. Association of CD34 expression with benign phyllodes tumor of breast is statistically significant. p value is < 0.05

10. Inverse correlation was found between stromal CD117 expression and CD34 Stromal expression, p value was < 0.05.

As the stromal expression CD117 increases, CD34 positivity decreases

11. Coexpression of both markers CD117 and CD 34 was found in 50% of borderline tumors. Association of CD 34 and CD117 expression with borderline phyllodes tumors is statistically significant. P value<0.05

12. Epithelial CD117 positivity was found in 45% cases of benign Phyllodes tumors of breast. Association of CD117 epithelial expression with benign phyllodes tumor of breast is statistically significant. p value is < 0.05

CONCLUSION

CONCLUSION

Our findings show that CD34 and CD117 markers are differentially expressed in benign and malignant PTs. CD34 was preferentially expressed in the stroma of benign phyllodes tumors. In contrast CD117 was preferentially expressed in the stroma of malignant PTs. Our results suggest that these markers might be used for the diagnosing the various histopathological grades of PT.

A larger study of PTs will be useful to accurately evaluate the significance of immunohistochemical markers with respect to histological grade and clinical outcome and to further understand the biological behavior and tumor progression.

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ANNEXURES

ANNEXURE I

PROFORMA

Name : Age :

Ward : IP NO:

Address :

Presenting Complaints

Lump in breast :

Pain :

Discharge from nipple :

Skin ulceration :

Duration of presenting illness :

Past history :

History of previous surgeries for breast lump :

History of chemotherapy/radiotherapy :

History of breast lump in other breast :

Family history : Personal history :

Diet : Menstrual history :

Breast feeding history :

General Examination

Nourishment : Built :

Conscious Febrile/afebrile : Pallor :

Jaundice : Cyanosis :

Clubbing : Lymphadenopathy :

Edema : Vitals :

PR : RR :

BP :

Local Examination of The Breast

Side – right/left :

Quadrant :

Size of the tumor :

Fixity to the skin :

Fixity to the underlying fascia :

Examination of Axillary Lymph Node

Number of node : Mobile/fixed :

Size :

Group of node : anterior/posterior/lateral/apical

Gross Examination of Modified Radical Mastectomy Specimen

Size of the specimen including skin, nipple, areola :

Size of the tumor :

Margins: infiltrative/circumscribed :

Quadrant :

Histological diagnosis :

Lymph node status – no: of positive nodes/no: of total nodes : examined

Histological grading

Stromal cellularity- mild/moderate/marked

Nuclear pleomorphism- mild/ moderate/ marked

Stromal overgrowth- present/absent

Mitotic rate- 0-2/hpf, 2-5/hpf, >5/hpf

Margins - circumscribed/invasive

ANNEXURE - II

GLOSSARY

CD	:	Cluster of Differentiation
DAB	:	Diaminobenzidine
DPX	:	Dextrene polystyrene xylene
ER	:	Estrogen receptor
GIST	:	Gastrointestinal stromal tumor
PCR	:	Polymerase chain reaction
PT	:	Phyllodes tumor
TBS		Tris buffer solution
MDM2	:	Mouse double minute 2 homolog
MYC	:	Avian myelocytomatosis viral oncogene homolog

ANNEXURE III

CONSENT FORM

Dr.R.ARTHI postgraduate student in the department of pathology, Coimbatore Medical College is conducting a study on **"STUDY OF EXPRESSION OF CD 117 AND CD 34 IN PHYLLODES TUMOR OF BREAST AND ITS CORRELATION WITH HISTOPATHOLOGICAL GRADE"**. Lumpectomy, mastectomy specimens are received in pathology department and are processed and examined under a microscope to obtain diagnostic information or is tested for other studies. I have been informed, to my satisfaction regarding the nature of procedure. The data used herein may be used for research and publication.

Name:

Place:

Signature:

ஒப்புதல் படிவம்

பெயர் .

வயது .

பாலினம் .

முகவரி .

அரசு கோவை மருத்துவக் கல்லூரியில் நோய் குறியியல் மருத்தவ துறையில் பட்ட மேற்படிப்பு பயிலும் மாணவி **மரு.இரா.ஆர்த்தி** அவர்கள் மேற்கொள்ளும் **"மார்பக கட்டியில் CD34 மற்றும் CD117 வின் வெளிப்பாட்டினை அறிதல்"** என்பதின் செய்முறை மற்றும் அனைத்து விளக்கங்களையும் கேட்டுக் கொண்டு எனது சந்தேகங்களை தெளிவுப்படுத்திக் கொண்டேன் என்பதை தெரிவித்துக் கொள்கிறேன்.

நான் இந்த ஆய்வில் முழு சம்மதத்துடனும், சுய சிந்தனையுடனும் கலந்து கொள்ள சம்மதிக்கிறேன்.

இந்த ஆய்வில் என்னைப் பற்றிய அனைத்து விவரங்கள் பாதுகாக்கப்படுவதுடன் இதன் முடிவுகள் ஆய்விதழில் வெளியிடப்படுவதில் ஆட்சேபனை இல்லை என்பதை தெரிவித்துக் கொள்கிறேன். எந்த நேரத்திலும் இந்த ஆய்வில் இருந்து நான் விலகிக் கொள்ள எனக்கு உரிமை உண்டு என்பதையும் அறிவேன்.

இடம்

தேதி கையொப்பம் / ரேகை

ANNEXURE IV

MASTER CHART

S.NO	HPE NO	AGE	HISTOPATHOLOGICAL GRADING						CD117		CD34
			STROMAL CELLULARITY	NUCLEAR PLEOMORPHISM	STROMAL OVERGROWTH	MITOTIC RATE/10hpf	MARGIN OF TUMOR	GRADE	STROMA	EPITHELIUM	STROMA
1	881/14	33	marked	marked	present	>5	invasive	malignant	positive	Faintly positive	negative
2	1561/14	36	marked	marked	present	>5	invasive	malignant	negative	negative	negative
3	1828/14	33	mild	mild	absent	nil	circumscribed	benign	negative	positive	negative
4	2752/14	48	mild	mild	absent	1 to 2	circumscribed	benign	negative	positive	positive
5	2754/14	58	mild	mild	absent	nil	circumscribed	benign	negative	negative	negative
6	2946/14	48	marked	marked	present	>5	invasive	malignant	positive	Faintly positive	negative
7	2980/14	56	marked	marked	present	>10	invasive	malignant	positive	negative	negative
8	3223/14	44	moderate	moderate	present	2 to 5	circumscribed	borderline	negative	negative	negative
9	3263/14	40	mild	nil	absent	nil	circumscribed	benign	negative	negative	negative
10	3487/14	39	moderate	moderate	present	3 to 4	circumscribed	borderline	positive	negative	positive
11	648/15	55	marked	marked	present	>5	invasive	malignant	negative	negative	negative
12	1553/15	20	mild	nil	absent	nil	circumscribed	benign	negative	positive	positive
13	1859/15	45	moderate	moderate	present	2 to 5	circumscribed	borderline	positive	negative	positive

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14	2230/15	57	moderate	moderate	present	2 to 3	invasive	borderline	positive	negative	positive
15	2252/15	48	marked	marked	present	>5	invasive	malignant	negative	negative	positive
16	2287/15	35	mild	moderate	absent	nil	circumscribed	benign	negative	negative	positive
17	2862/15	32	mild	nil	absent	1 to 2	circumscribed	benign	negative	negative	positive
18	944/16	40	moderate	moderate	present	2 to 5	circumscribed	borderline	negative	positive	positive
19	1465/16	37	mild	mild	absent	nil	circumscribed	benign	negative	positive	positive
20	1641/16	55	marked	marked	present	>5	invasive	malignant	positive	negative	negative
21	E115/16	45	mild	mild	absent	nil	circumscribed	benign	negative	negative	positive
22	1777/16	42	mild	mild	absent	nil	circumscribed	benign	negative	negative	positive
23	1823/16	44	moderate	moderate	present	2 to 3	circumscribed	borderline	positive	negative	negative
24	1899/16	41	marked	marked	present	>5	invasive	malignant	negative	negative	positive
25	2001/16	42	mild	nil	absent	nil	circumscribed	benign	negative	positive	positive